BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. Conklin—*Princeton University.*

Jacques Loeb—*The Rockefeller Institute for Medical Research.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Volume XXVII.

WOODS HOLE, MASS.
JULY TO DECEMBER 1914

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

CONTENTS OF VOLUME XXVII

No. 1. JULY, 1914.

LOEB, LEO. *The Correlation Between the Cyclic Changes in the Uterus and the Ovaries in the Guinea-pig* 1

HOY, WILLIAM E., JR. *A Preliminary Account of the Chromosomes in the Embryos of Anasa tristis and Diabrotica vittata* 45

ALLEE, W. C. *The Ecological Importance of the Rheotactic Reaction of Stream Isopods* 52

No. 2. AUGUST, 1914.

RICHARDS, A. *The Effect of X-Rays on the Rate of Cell Division in the Eearly Cleavage of Planorbis* 67

WELCH, PAUL S. *Habits of the Larva of Bellura Melanopyga Grote (Lepidoptera)* 97

FASTEN, NATHAN. *Fertilization in the Parasitic Copepod, Lernæopoda Edwardsii Olsson* 115

No. 3. SEPTEMBER, 1914.

ALLEN, WM. RAY. *The Food and Feeding Habits of Freshwater Mussels* 127

GOODRICH, H. B. *The Maturation Divisions in Ascaris incurva* 147

CHAMBERS, ROBERT, JR. *Linkage of the Factor for Bifid Wing. The Bifid Wing and Other Sex-linked Factors in Drosophila* 151

EWING, H. E. *Notes on the Regression in a Pure Line of Plant Lice* 164

No. 4. OCTOBER, 1914.

HEWITT, J. H. *Regeneration of Pleurotricha after Merotomy with Reference Especially to the Number of Micronuclei and the Occurrence of Uninucleate Cells* 169

POWERS, EDWIN B. *The Reactions of Crayfishes to Gradients of Dissolved Carbon Dioxide and Acetic and Hydrochloric Acids* .. 177

JUST, E. E. *Breeding Habits of the Heteronereis Form of Platynereis megalops at Woods Hole, Mass.* 201

CASE, E. C. *On the Structure of the Inner Ear in Two Primitive Reptiles* 213

FOOT, KATHARINE, AND STROBELL, E. C. *Preliminary Report of Crossing two Hemipterous Species with Reference to the Inheritance of a Second Exclusively Male Character*..... 217

No. 5. NOVEMBER, 1914.

HARVEY, E. NEWTON. *Is the Fertilization Membrane of Arbacia Eggs a Precipitation Membrane?*..... 237
KINGERY, H. M. *So-called Parthenogenesis in the White Mouse*.... 240
BANTA, A. M. AND GORTNER, R. A. *A Milky White Amphibian Egg Jell*..... 259
ROGERS, C. G. AND LEWIS, E. M. *The Relation of the Body Temperature of the Earth-worm to that of its Environment*..... 262
ROGERS, C. G. AND LEWIS, E. M. *The Temperature Coefficient of the Rate of Contraction of the Dorsal Blood-vessel of the Earth-worm*..... 269
TURNER, C. H. AND SCHWARZ, E. *Auditory Powers of the Catocala Moths; an Experimental Field Study*..... 275

No. 6. DECEMBER, 1914.

WODSEDALEK, J. E. *Spermatogenesis of the Horse with Special Reference to the Accessory Chromosome and the Chromatoid Body*.. 295
TURNER, C. H. *An Experimental Study of the Auditory Powers of the Giant Silkworm Moths (Saturniidæ)*..... 325
HICKERNELL, L. M. *A Preliminary Account of Some Cytological Changes Accompanying Desiccation*..... 333
RUNYAN, E. M., AND TORREY, H. B. *Regulation in Vorticella*..... 343

Vol. XXVII. *July, 1914.* *No. 1*

BIOLOGICAL BULLETIN

THE CORRELATION BETWEEN THE CYCLIC CHANGES IN THE UTERUS AND THE OVARIES IN THE GUINEA-PIG.[1]

LEO LOEB.

TABLE OF CONTENTS.

I. The Cyclic Changes in the Uterus of Guinea-pigs in which Copulation Takes Place..... 1
II. The Sexual Cycle of Uterus and Ovaries in Guinea-pigs in which Heat had been Observed, but Copulation Prevented..... 9
III. The Effect of Extirpation of the Corpora Lutea on the Cyclic Changes in the Uterus..... 17
IV. The Uterus of Pregnant Guinea-pigs in which after Excision of the Corpora Lutea a New Ovulation has Taken Place..... 22
V. The Influence of Extirpation of the Corpora Lutea on the Duration of the Cyclic Period..... 31
VI. The Signs of Heat in the Guinea-pig..... 32
VII. Effect of Extirpation of the Ovaries on the Cyclic Changes in the Uterus. 34
VIII. Summary..... 40

In former papers we analyzed the production of the maternal placenta and of the experimental placenta; we followed the cyclic changes in the mammalian ovaries and partly analyzed the factors determining the first step in these changes, namely ovulation. In this paper we shall describe more in detail the cyclic changes in the uterine mucosa of the guinea-pig and correlate these changes with those in the ovaries. We shall analyze the effect of pregnancy, of the corpora lutea and other parts of the ovaries on those changes.

I. The Cyclic Changes in the Uterus of Guinea-pigs in Which Copulation Takes Place.

At the period of heat and soon after copulation.—In the uterus the surface epithelium and the gland ducts show high cylindrical epi-

[1] From the Department of Pathology, Barnard Free Skin and Cancer Hospital, St. Louis, Mo.

thelium and there can be seen several rows of surface epithelium. The gland fundi have much lower and smaller cells. The number of mitoses in these cells varies. They may be absent or almost absent. Often there are mitoses present in the gland ducts and sometimes in the surface epithelium. They are always absent in the gland fundi. The connective tissue under the epithelium is rather cellular and fibrils may be missing, or be little pronounced; in the deeper connective tissue they are more prominent. Mitoses are usually lacking in the connective tissue, but a few may be present especially near the epithelium of the surface of the gland ducts. Some mononuclear cells and also a few polynuclear leucocytes can be seen in the mucosa and may migrate through the surface epithelium, or collect in its neighborhood. The uterus is usually slightly papillary. There is no marked hyperemia in the mucosa, which, however, is somewhat edematous. Some gland fundi are cystically dilated, and partly filled with leucocytes, partly without leucocytes.

Within the first hour after copulation the uterine epithelium (of the surface as well as of the gland ducts) is high cylindrical; in the gland fundi we find lower cuboidal cells; inasmuch as the quantity of cytoplasm is relatively small in the gland fundi, the blue color predominates if the sections are stained with haematoxylin and eosin. The glands as a whole are small, straight and widely separated. Mitoses are more or less frequent in the surface epithelium and in that part of the glands in which the cells are high cylindrical, the mitoses are, however, more frequent in the glands. The connective tissue is rich in cells, the nuclei are somewhat larger, the tissue is in parts somewhat edematous, especially towards the surface; no mitoses are seen in the connective tissue. Some gland ducts are filled with polynuclear leucocytes; these are also present around glands; many glands, however, are free from polynuclears. Small mononuclear cells migrate through the surface epithelium. One hour after copulation the condition of the mucosa is almost the same as five minutes after copulation; the number of the mitosse in the surface epithelium has perhaps been somewhat decreased. The mucosa is quite edematous; a mitosis is seen in the connective tissue (perhaps in an endothelial cell.) Some spermatozoa are

visible in the lumen of the uterus and of the glands. Leucocytes migrate through the mucosa into the glands.

Three, four and five hours after copulation, the surface epithelium is high cylindrical. Polynuclear leucocytes migrate through the surface epithelium and may injure epithelial cells of the surface epithelium; vacuoles may appear in the surface epithelium. As the result of the injurious effects of the leucocytes, the surface epithelial cells may become lower and their nuclei deformed and contracted. There are not any, or only a few mitoses in the surface epithelium. The gland ducts are high cylindrical; they appear with hæmatoxylin-eosin red-stained, the cytoplasm preponderating. The ducts are short, the gland fundi have lower cuboidal epithelium. In the gland ducts there are some mitoses present; they are missing in the gland fundi where the cells are cuboidal. Polynuclear leucocytes migrate also through gland epithelium into the glands and may occasionally injure the gland epithelium. The connective tissue shows a distinct fibrillar character; its nuclei are rather large; it is somewhat edematous; some leucocytes migrate from the vessels through the connective tissue towards the epithelium. There are a few mitoses in endothelial cells of blood vessels and possibly also in connective tissue cells. The uterus shows blunt papillae. The sperm fluid present in the lumen of the uterus exerts a pressure on the surface epithelium and may thus contribute to the harmful influence of the leucocytes. In a specimen eight hours after copulation, the same results were obtained. There were, however, numerous mitoses in the surface epithelium.

It seems, therefore, as if the number of mitoses in the surface epithelium a few hours after copulation was determined by accidental conditions such as the action of leucocytes and pressure of the fluid, and was, therefore, variable to the same extent as these factors varied. The leucocytes may pass in such quantities through the mucosa into the lumen of the uterus that the fluid in the uterine cavity appears like an abscess and the leucocytes almost destroy the surface epithelium at certain places. Agglutinated spermatozoa are seen in the lumen of the uterus a few hours after copulation, and spermatozoa are also seen in the gland ducts. The polynuclear leucocytes migrate

in large numbers through the connective tissue of the mucosa, through the epithelium and the gland ducts. Under their influence much of the surface epithelium has been destroyed and even the epithelium of the gland ducts has been loosened.

Soon after parturition the guinea-pig is ready to copulate. At this time the mucosa is papillary and low. The muscle tissue lies at a number of places near the surface epithelium; the glands are therefore short; the epithelium is cuboidal or cylindrical, at some places high and with much cytoplasm; the vesicular nuclei are often at the base of the cell. Sometimes there are vacuoles in the epithelial cells. Mitoses are extremely rare in the epithelium; the gland cells are small and without mitoses. The connective tissue in the mucosa is somewhat edematous; it forms only a small layer; the nuclei are not large, rather densely packed; mitoses are not visible. At some places the epithelium is absent and coagulated blood with polynuclear leucocytes covers the surface. The muscle tissue is turgid and some plasmodia are visible around the vessels of the muscular coat. The condition of the surface epithelium is here apparently similar to the epithelium during the period of heat.

From twelve to twenty-four hours after copulation.—This period is marked through the great number of polynuclear leucocytes seen in the lumen of the uterus, in the lumen of the glands and especially in the upper ducts of the glands. There may be also present abscess-like collections of polynuclear leucocytes in the connective tissue of the mucosa. They may raise up the surface epithelium from the underlying connective tissue at some places; at other places they rarify and almost destroy the epithelial cells. We may still see twelve hours after copulation the high cylindrical shape of these cells. The glands are short and they do not show much branching; the gland ducts still show a lining of cylindrical cells; some gland-lumina are dilated through masses of leucocytes and the leucocytes may exert such a pressure on the walls of the glands that the epithelium becomes flat. Mitoses are seen at this period.

From one to four days after copulation.—Two days, five and a half hours, and two days, seven hours after copulation, the epithelium of the surface and of the gland ducts is cylindrical

and usually high; the gland fundi do not show as high an epithelium. There are mitoses present in the surface epithelium and in the upper and medium parts of the glands; but the mitoses may reach also further into the lower part of the glands. The gland fundi are not large nor separated from each other by much connective tissue. The connective tissue of the mucosa contains many nuclei; the fibrils are not pronounced and the tissue has a succulent, somewhat myxoid appearance; the nuclei are vesicular. Between the gland fundi the connective tissue is more fibrillar. No mitoses are as yet visible in the connective tissue of the mucosa; some leucocytes are still to be found there. We see, therefore, that the large masses of leucocytes have mostly disappeared as early as two days after copulation, and that the damage done to the epithelial structure has been repaired.

In the first half of the fourth day after copulation the epithelial structures are on the whole unchanged; the surface and gland duct epithelium is cylindrical, but at the surface there may be some cuboidal cells; the gland fundi are still composed of smaller cells, but in the second half of the day, the gland fundi also become active and show frequent mitoses. In the surface epithelium there are at this period either none at all, or only exceptional mitoses. At this period begins also the increase in the number of connective tissue cells in the mucosa. Three days, eight hours after copulation, the connective tissue of the mucosa has a myxoid character; the nuclei of the connective tissue cells are still small, but a little larger than at former periods; only exceptionally a mitosis is visible in the connective tissue of the mucosa. Many blood vessels are present and the tissue appears hyperemic, but no mitoses are seen in the vessels. The connective tissue between the fundi is denser. Three days and twelve hours after copulation, the mucosa is wider; there are round or oval vesicular nuclei present in the connective tissue cells; their cytoplasm is permeated with vacuoles; a certain though not yet large number of mitoses is seen in the connective tissue cells and these mitoses are independent of mitoses in endothelial cells of blood vessels which also occur. Thus the connective tissue cells become gradually transformed into predecidual cells. The connective tissue assumes a similar structure as regenerating

connective tissue in wounds, the vessels running at right angles to the bundles of connective tissue cells. The enlargment of the connective tissue causes a drawing out of the gland ducts and now mitoses appear also in the gland fundi, perhaps as a result of the pull exerted on the gland ducts. Some leucocytes migrate through the mucosa and epithelium.

About four days after copulation, or soon afterwards, the surface epithelium has become lower; it is either cuboidal or cylindrical, of medium height. No mitoses are present in the surface epithelium or in the cuboidal or low cylindrical gland ducts. The cells in the fundi of the glands are cylindrical and there are very many mitoses present. The mitoses migrate therefore gradually from the surface into the gland fundi, and concomitantly with the decrease in mitoses we find a decrease in the size and shape of the epithelial cells of the surface and gland ducts. In the connective tissue there is now a decided increase in the number of mitoses especially in cells near the surface and gland epithelium. The connective tissue cells themselves have vesicular nuclei and form fibrillar processes. Between the gland fundi the intercellular fibrils are more marked; the number of nuclei is smaller and there are less mitoses present. The tissue may, however, be edematous. The mitoses are mainly present in the fibroblasts, but there are some mitoses also in endothelial cells of capillaries. The proliferation takes place principally in the fibroblasts and the predecidual cells do not originate in the endothelium of the blood vessels. The vessels are often running directly towards the surface epithelium and at right angles to the direction of the connective tissue cells. There are occasionally some polynuclears present in alveoli of glands.

Six to eight days after copulation.—Six days, and on the seventh day after copulation, the surface and the gland epithelium is cuboidal low, or medium cylindrical. The gland ducts are drawn out. In the glands there may still be some higher cylindrical epithelial cells left; nowhere are mitoses present in the epithelium. There are mitoses in the connective tissue underneath the surface epithelium and between the gland ducts. The arrangement is the one typical for regenerating connective tissue, the vessels running at right angles to the layers of the

connective tissue. Below the epithelium there are layers of densely packed large vesicular nuclei; the cells are often free, not enveloped by fibrils. The number of mitoses varies somewhat at different places. The gland fundi are surrounded by edematous connective tissue and between the gland fundi we find fibrillar connective tissue. The cellular connective tissue separates the gland fundi from the surface epithelium. Some polynuclear leucocytes are in the mucosa; we see here some disintegrating cells; it is possible that they are mainly polynuclear leucocytes.

Seven days, and on the first half of the eighth day after copulation, the surface epithelium is cuboidal or low-medium cylindrical; at a few places it is somewhat higher with vacuoles in the outer part of the cells; the nuclei are small vesicular. Usually no mitoses are present, but in one piece a few mitoses could be seen.

The epithelium of the glands is cuboidal or low cylindrical; the glands are drawn out. In one piece there are still a number of mitoses in the gland fundi, although the gland cells are here cuboidal. The structure of the connective tissue is similar to that of the previous period. There are mitoses present in the cell layer but they are not quite as frequent as before. The cell layer consists of round, relatively small cells with many capillaries with narrow endothelium. There is some hyperemia present. At other places the nuclei in the cell layer are round vesicular, and the cytoplasm of the neighboring cells is connected by bridges.

Eight days, eight hours, after copulation, the structure of the surface epithelium and glands is similar to the previous period. The epithelial cells are low. No mitoses are present. The glands are not very large and contain a relatively small number of coils. There is still some cell layer present under the surface epithelium; the vesicular nuclei are compressed and a number of nuclei under the epithelium disintegrate, perhaps the nuclei of the proliferated connective tissue layer.

Eight to seventeen days after copulation.—In order to prevent pregnancy the tubes had been ligatured near the junction with the uterus soon after copulation.

Ten to eleven days after copulation.—The surface epithelium is lower than at the beginning of the sexual cycle; it is cuboidal or low cylindrical; at certain places there may be swollen cells. There are generally no vacuoles in the epithelium; mitoses are usually missing; they were present only in one place. The glands are small coiled, lying near the surface, are without a wide lumen, their cells are not high and without mitoses. The connective tissue of the mucosa is well supplied with capillaries, which run at right angles to rows of connective tissue cells. The connective tissue is still partly myxoid under the surface of the epithelium. At some places there are still many vesicular nuclei densely packed. In the deeper connective tissue between the coils of the glands there are smaller nuclei and some fibrils. Only one mitosis is seen in a connective tissue cell in the mucosa. There is some nuclear debris present under the epithelium; some mononuclears are in the mucosa tending to migrate through the epithelium.

Twelve to fourteen days after copulation.—Low cuboidal or low cylindrical epithelium of surface with *frequent* mitoses, especially at places of invagination of the epithelium; glands with low cuboidal or low cylindrical epithelium without mitoses. In the connective tissue there are no mitoses; small vesicular nuclei of connective tissue cells under the epithelium of the surface. The further the connective tissue is removed from the surface epithelium, the more the fibrils become developed and the smaller the nuclei. There is occasionally a little edema in the connective tissue. Some small mononuclear cells may migrate through the epithelium. The layer of proliferated connective tissue cells which was previously present is much smaller now than five to seven days after ovulation. The glands are correspondingly smaller. At this period mitoses appear again in the surface epithelium.

Fifteen to seventeen days after copulation.—The epithelial cells are low cuboidal; mitoses are present only in the surface epithelium. The connective tissue contains small nuclei and is fibrillar. Near the surface epithelium the nuclei are relatively more frequent and vesicular in the connective tissue, and the fibrils are less developed than in the deeper parts of the mucosa.

Occasionally some edema is present in the surface epithelium and at such places vacuoles are present in the epithelial cells.

This condition of the uterus may exist notwithstanding the presence of follicles in the ovaries which are near maturity. The latter do, therefore, at least as long as the corpora lutea functionate, not produce the changes in the mucosa of the uterus which are characteristic of the first period in the sexual cycle.

During pregnancy the uterus below or above the insertion of the placenta or in the horn in which there happens to be no embryo shows low cuboidal or low cylindrical epithelium with or without mitoses. There may be some vacuoles in the epithelial cells. There are small narrow coiled glands with low cylindrical epithelium. There is no large connective tissue cell layer present. The nuclei in the connective tissue of the mucosa are small and surrounded by well developed connective tissue fibrils; especially between the gland fundi do we find large connective tissue fibers, while underneath the epithelium the number of connective tissue nuclei is relatively greater and the tissue is less fibrillar. Mitoses occur neither in the glands nor in the connective tissue. It seems that in the earliest stages of pregnancy less than ten days after copulation, there are not any or only exceptional mitoses in the surface epithelium; correspondingly we saw previously that six to ten days after a previous period of heat—copulation having been prevented—no mitoses were found in the surface epithelium. During the middle or end of pregnancy the uterine mucosa has a similar structure; but there may be present some papillæ in the mucosa. The presence of mature, or nearly mature follicles in the ovaries is without influence on the structure of the uterus during pregnancy.

II. The Sexual Cycle of Uterus and Ovaries in Guinea-Pigs in Which Heat had been Observed but Copulation Prevented, is as Follows:

At the period of heat previous to the rupture of the follicles we find the following condition: In the ovaries we find one or more small corpora lutea which are just beginning to degenerate (vacuolization in the periphery). The follicles with exception of the smallest show degeneration of the granulosa. There may

be at earlier stages still some good medium-sized follicles. There are present one or several mature follicles usually with maturation spindle in the ovum of the follicle. Other follicles show early and medium stages of connective tissue atresia. There are usually many follicles in or near the last stage of connective tissue atresia, but the number of such follicles varies and may be relatively small in very young ovaries at the time of the first heat. The structure of the uterus at this period has been described above (see page 1). It is identical with the structure of the uterus in the period of heat when the latter is followed by copulation. Even after extirpation two months previously of the greater part of the uterus, the same phenomena can be observed; heat takes place and mature follicles form. The remaining small part of the uterus forms a little cyst with cylindrical (or cuboidal) epithelium with many mitoses. The gland ducts have highly cylindrical epithelium and also show mitoses. This has been observed in two experiments. Also after almost complete extirpation of the thyroid one to two months previously, heat occurs accompanied by the typical changes in the uterus and ovaries. The small remaining part of the thyroid may consist of acini with cylindrical epithelium while the colloid may be almost, or entirely absent.

At a slightly later period, namely, soon after the rupture of the mature follicles, the condition of the ovaries and uterus is essentially the same; two changes have however, taken place, namely (1) the surface epithelium is not quite so regularly high cylindrical, but may be at certain parts cuboidal; and (2) we find more or less pronounced changes in the surface epithelium and sometimes in the adjoining part of the gland ducts, namely, vacuolization in the epithelial cells; in the vacuoles which press upon the nuclei we may find degenerating cells; chromatin particles may spread over the greater part of the cells. These changes are probably due to the immigration of mononuclear cells or polynuclear leucocytes from the mucosa into the epithelium. Here these immigrated cells degenerate. One sees sometimes mononuclear cells immigrating into the epithelium. It is not very probable that the changes in the epithelium following ovulation are brought about through a disintegration of some epithelial cells.

Mitoses are very rare in the epithelial structures; there may be however a very small number present in the surface epithelium or in the upper gland ducts. Some glands at this period are dilated and filled with polynuclears. Only rarely do we see a mitosis in the connective tissue. We find in some pieces soon after the rupture of the mature follicles hemorrhages beneath the epithelium which may raise the surface layer of the epithelium from the underlying connective tissue.

The same condition we find also in the course of the first day after the rupture of the follicles, at a time, therefore, when connective tissue begins to grow into the follicular cavity. Perhaps the degenerative changes in the surface epithelium are even more marked at this period.

In four animals in which the ovaries and uterus were examined seventeen and eighteen days after a previous heat, we found ruptured follicles in the animals examined (after seventeen days) or very young corpora lutea (after eighteen days) and the corresponding condition of the uterine mucosa.

We may therefore conclude that in animals kept under normal conditions the period of heat occurs approximately every fifteen to nineteen days, a conclusion that is corroborated by other observations which we made. We furthermore conclude that the condition of the uterine surface epithelium indicates in most cases whether or not a rupture of a follicle has taken place in the ovary.

In a specimen obtained *about one to one-and-a-half days after spontaneous ovulation*, in which no copulation had taken place, there were present at most places high cylindrical epithelium of the surface; at some places, however, the epithelium was somewhat lower; gland fundi show as usual lower epithelium. In the surface epithelium and in the gland ducts with higher epithelium there are mitoses present. The mucosa is rich in cells; is partly a little edematous; there is no increase in the size of the mucosa visible and no mitoses are seen. Some polynuclear leucocytes are present in the mucosa, connective tissue as well as glands, although no spermatozoa had entered the uterus; but the number of leucocytes is much smaller than in cases in which copulation had taken place.

Three days after heat we find a vacuolar corpus luteum of the former period, in process of degeneration. In the central cavity of the new corpora lutea new connective tissue is present in the periphery. There are many small, and small to medium-sized good follicles; many follicles in an early-medium stage of connective tissue atresia, and a large number of follicles in the late stages of connective tissue atresia.

The surface epithelium of the uterus is mostly high cylindrical, but may be at places cuboidal, probably as a result of stretching. It does not show any longer the changes observed in the first two days after ovulation. It is usually free from mitoses; the gland duct epithelium is high cylindrical and the epithelium of some fundi begins to become also higher. There are many mitoses present in the gland ducts, especially in their medium parts; the mitoses begin now to proceed further downwards and occasionally may be found in these gland fundi which have enlarged. The connective tissue of the mucosa is, especially in the higher parts, rather cellular, with vesicular nuclei, but without mitoses; it may be somewhat myxoid.

In the mucosa we find some small mononuclear cells and also some polynuclear leucocytes. Some mononuclears may immigrate into the gland and surface epithelium. There may be seen some cystically dilated glands.

Four days after heat the corpus luteum of the former period is vacuolar; the new follicles are well developed. There are many small and medium-sized good follicles, and many follicles in medium or the last stage of connective tissue atresia. The epithelium of the surface and gland ducts is no longer as high as before; it is cylindrical, of medium height, or cuboidal-cylindrical; the epithelium of the gland fundi is higher than before. There are many mitoses present in the gland fundi; but nowhere else in the epithelium. Some glands are dilated and contain polynuclear leucocytes. The connective tissue layer under the epithelium is enlarged and shows frequent mitoses. The uterus is slightly papillomatous and in the connective tissue of the papillæ there is some edema. The epithelium over the edematous connective tissue may also be edematous.

Five days after heat, we find in the ovaries many small and

medium-sized good follicles, a number of follicles in a medium stage of connective tissue atresia, and there are a large number of follicles in the last stage of connective tissue atresia. The surface and glandular epithelium is cylindrical, of medium height and is almost without mitoses; a few isolated mitoses may be found in the gland fundi. The cell layer of the connective tissue contains many mitoses; there are some small mononuclear, and a few polynuclear leucocytes in the mucosa.

Six days after heat, the corpora lutea of the former period are quite vacuolar and in process of degeneration. There are good new corpora lutea and many good small, medium-sized and large follicles, and many follicles in the last stages of connective tissue atresia. The surface epithelium of the uterus is cylindrical and of medium height; the epithelium of the gland ducts and gland fundi is cuboidal-cylindrical. There are nowhere mitoses present in the epithelium. There are mitoses present in the connective tissue cell layer of the mucosa, but the mitoses are here probably not as frequent as at five days after the previous period of heat. There are many small mononuclear cells in the connective tissue.

Seven days after heat, we find good corpora lutea, many good small medium-sized and large follicles, no follicles with granulosa degeneration and many follicles in the last stage of connective tissue atresia. The epithelium of the surface and glands of the uterine mucosa is cuboidal or cylindrical of medium height. No mitoses are present in the epithelium; some mononuclears can be found in the surface epithelium. In the connective tissue cell layer there are a number of mitoses, but not as many as five days after heat. Some polynuclear leucocytes and small mononuclear cells are in the mucosa and some nuclei disintegrate in the upper layer of the connective tissue.

Eight days after heat, the condition of the ovaries is the same as seven days after the period of heat, but degeneration of the granulosa has now set in in some of the larger follicles. . The corpora lutea of the former period are quite vacuolar and are in full process of retrogression. The epithelium of surface and glands of the uterus is cuboidal or cylindrical of medium height, without mitoses. In the connective tissue of the mucosa we still see blood vessels running towards the surface and at right

angles to the long axis of the connective tissue cells; but mitoses are no longer present and some cells disintegrate in the upper part of the mucosa.

Nine days after heat.—There is as yet not any, or very little granulosa degeneration in the large follicles; occasionally there may be more extensive degeneration of the granulosa and a few exceptional follicles may show an early stage of connective tissue atresia; in some ovaries all the large follicles are still well preserved. The follicles which six to eight days after the former period of heat were in the last stage of connective tissue atresia, shrink under the influence of the pressure of the growing follicles and corpora lutea.

The uterine epithelium of the surface is low to medium-sized cylindrical, a few vacuoles may be present in the epithelium, the gland epithelium is low cylindrical. The connective tissue of the mucosa shows still vesicular nuclei and vacuoles in the cells, but it is being somewhat compressed. There are no mitoses present in the epithelium or in the connective tissue.

Eleven days after heat.—We find good small, medium-sized and large follicles, some large follicles show granulosa degeneration or connective tissue in growth. The follicles which were formerly in the last stage of connective tissue atresia begin to disappear more and more; there are still the remains of the membranæ pellucidæ, surrrounded by some very small compressed nuclei. The corpora lutea are in a good condition. Mature follicles appear again in the ovaries.

The epithelium of surface and gland ducts of the uterus is cyclindrical, of medium size. The gland fundi are smaller. No mitoses are seen; some mononuclear cells may be present, but neither vacuoles nor chromatin particles; there is still some remnant of the cell layer in the mucosa, but it is becoming smaller and more fibrillar; only an exceptional mitosis can be found in the connective tissue.

We find, therefore, active cell proliferation in the connective tissue of the mucosa from the fourth to the seventh day after the last ovulation. Approximately five days after ovulation the mitoses disappear also in the gland fundi. From the sixth to the twelfth day after ovulation, the epithelium of the uterine mucosa is without mitoses. Then they begin to reappear.

Twelve days after heat.—There are many good small, medium-sized and large follicles; some large follicles show granulosa degeneration. A number of follicles show early and medium and some advanced connective tissue atresia. The follicles which had arrived about six to seven days after copulation at the last stage of connective tissue atresia are disappearing more and more. Some mature follicles may be present.

The surface and glandular epithelium of the uterus is cuboidal-cylindrical; some small mononuclear cells migrate through the surface epithelium. No definite mitoses can be seen in the surface epithelium. A few isolated gland ducts and the adjoining surface epithelium have high cylindrical epithelium. The mucosa is narrow and the nuclei of the connective tissue are small.

Fourteen days after heat.—In the corpora lutea some vacuolization begins in the periphery. There are good, small medium-sized and large good follicles, some large follicles show granulosa dgeneration; other follicles are in the early or medium or a later stage of connective tissue atresia. These follicles which are now constantly degenerating all contribute to the follicles in the late stages of connective tissue atresia which we find at the time of heat.

The surface and gland duct epithelium of the uterus is cuboidal or cylindrical of medium height. The gland fundi are smaller. There are some mitoses present in the surface and gland duct epithelium. The mucosa is low, the connective tissue nuclei are small. There is some edema present in the connective tissue which may even raise up the surface epithelium. There may be some dilated glands with polynuclear leucocytes and some mononuclears in the mucosa.

Fifteen days after heat.—At this time the ovaries are about the same as fourteen days after heat. There are good small, medium-sized and large follicles; a number of large follicles show granulosa degeneration. A certain number of follicles are in the medium- or medium-late stages of connective tissue atresia. There are not many follicles in the last stage of connective tissue atresia. The follicles which degenerated at the period of the last heat have almost disappeared and the other degenerating follicles

have not yet advanced to the last stage of atresia. There are mature follicles present. The periphery of the corpora lutea show some slight vacuolization. The connective tissue of the uterine mucosa is at this period very edematous, the edematous fluid may raise the surface epithelium. The connective tissue nuclei are very small. The mucosa and also the muscularis of the uterus are thin. The epithelium of the surface and of the gland ducts is low cuboidal or low cylindrical. This is perhaps partly due to the pressure of the underlying edematous fluid. Where the connective tissue is very edematous, the overlying epithelial cells may also be somewhat edematous and vacuolar. At some places the epithelium of the surface and gland ducts is somewhat higher. Some mitoses are present in epithelial cells of the surface and at the entrance of the gland ducts. Exceptionally a mitosis may be found in a gland duct.

We see, therefore, that from the fourteenth day on, mitoses reappear in the surface epithelium of the uterine mucosa. If we compare the cyclic changes in the uterus in cases in which copulation took place and in which only the heat was observed, but copulation had been prevented, we find the following:

A few hours after copulation, the leucocytes begin to migrate in large numbers from the connective tissue of the mucosa through the surface epithelium into the lumen of the uterus. Here they collect in such large numbers eight hours after copulation that its contents may be like an abscess.

These leucocytes exert an injurious influence on the surface epithelium which is therefore after copulation very much earlier and very much more strongly injured than if copulation has been prevented. The epithelium of the gland ducts may also suffer. We find, a few hours after copulation, spermatozoa which may be agglutinated in the lumen of the uterus and also in the gland lumen. The same large number of polynuclear leucocytes is still present during the first twenty-four hours after copulation; they have however mostly disappeared two days after copulation. At that time the condition of the uterus is very similar in animals in which copulation took place and in which copulation had been prevented. A few leucocytes can also be seen in the uterus of animals in which copulation had been prevented. In such

animals also some degenerative changes occur in the uterine epithelium, but they are less marked than in animals which had copulated. An examination of the uterus permits us in most cases to determine whether or not ovulation had taken place at the time of the examination in a guinea-pig in which copulation had been prevented.

From now on, the condition of the uterus is similar in both series of guinea-pigs; the mitoses migrate from the surface epithelium to the gland fundi and disappear completely in the epithelium five to six days after the ovulation. The connective tissue proliferation starts on the fourth day and ceases seven days after ovulation. On the eighth day we find a beginning disintegration of cells in the upper part of the connective tissue of the mucosa. The later stages were also examined in the first series in animals in which after copulation, pregnancy had been prevented through an early ligature of the tubes. From the sixth to the twelfth day after ovulation the epithelium is without mitoses; they then begin to reappear in the surface epithelium. Notwithstanding the presence of mature follicles, the uterus does not assume the characteristic features of the uterus during heat. These changes occur only after signs of degeneration have begun to set in the corpora lutea.

During pregnancy the uterine epithelium is relatively low, mitoses occur only in the surface epithelium. The connective tissue layer is thin and no mitoses are found in the connective tissue cells. The uterus is therefore in a resting condition during pregnancy.

III. The Effect of Extirpation of the Corpora Lutea on the Cyclic Changes in the Uterus.

In a previous communication we have shown that extirpation of the corpora lutea within the first week after ovulation leads in the majority of cases to an acceleration of the next ovulation. In nine cases in which pregnancy was not present at the time of the examination, the ovulation had not yet taken place notwithstanding the complete extirpation of the corpora lutea. In four of these cases the uterus had been split within the first week after ovulation. In another case abortion had taken place

at the time of the examination; in the sixth case the guinea-pig had been in a very weak condition and in the ovaries no good large or mature follicles, but some large follicles in granulosa degeneration, were found. In the seventh case the corpora lutea had been burnt instead of excised with the scissors. As we saw previously, the burning out of the corpora lutea injures the ovaries and prevents in many cases an early ovulation. In four cases in which the uterus had been cut, and in one other case, one ovary had been excised previously.

The uterus in these cases in which ovulation had not been hastened through extirpation of the corpora lutea was found to be as follows:

(1) No. 642. Three days after copulation extirpate corpora lutea examination. Sixteen days after the previous copulation the animal was in a weak condition. In the ovaries there was no good large or mature follicle, there were some large follicles with granulosa degeneration. The surface epithelium of the uterus was cuboidal; it contained some vacuoles with chromatin particles; there were very few mitoses present. The uterine glands were cuboidal to cylindrical and showed no mitoses. The connective tissue was fibrillar, the nuclei near the surface epithelium were more numerous; no mitoses were present in the connective tissue.

(2) No. 693. Seven days after the previous copulation extirpate the corpora lutea; ten days later examination. Abortion had taken place. The ovaries showed small and large good follicles and large follicles with granulosa degeneration; mature follicles were present. There were also follicles in various stages of connective tissue atresia.

The surface epithelium of the uterus was cuboidal to cylindrical and showed many mitoses; the glands were small and without mitoses; there was fibrillar connective tissue; where abortion had taken place, the connective tissue was hyperæmic, edematous and there were many polynuclears in the vessels.

(4) No. 300. Twenty-three hours after copulation extirpate left ovary; six days later make incision into the uterus; seventeen days after copulation, examine the uterus. In the remaining ovary of the animal which was about four months old, no corpus luteum and only small and small-medium sized good follicles

were found, besides follicles in an early and medium stage of connective tissue atresia. The uterus shows low cylindrical epithelium and glands with similar epithelium; some glands are dilated. There is a very small number of mitoses in the surface epithelium and in the superficial part of the gland ducts; the connective tissue is fibrillar.

The uterus is similar in No. 270 (four days after copulation excise left ovary, three days later cut out the uterus, examination seventeen days after copulation). In the ovary there was no mature follicle. The epithelium of surface and glands of the uterine mucosa was low, mitoses only present in the surface epithelium; the connective tissue of the mucosa was fibrillar and with small nuclei, without mitoses.

Similar were the findings also in No. 93 (three and a half days after copulation extirpate left ovary, one day later make incision in the uterus, examination fourteen and one half days after copulation). In this case there is however at some places somewhat higher surface and gland epithelium. In No. 992 (six days, ten hours after copulation one ovary was extirpated; the uterus was not cut; eighteen days ten hours after the previous ovulation, the examination was made). In this case the uterine epithelium was low cuboidal to cylindrical and contained mitoses; the glands were similar without mitoses, the connective tissue underneath the epithelium had some larger vesicular nuclei, and became fibrillar somewhat deeper.

In No. 843 the corpora lutea had been extirpated six days, sixteen hours after copulation; ovaries and uterus examined twenty and one half days after ovulation; there were many good medium-sized follicles present, others showed granulose degeneration, still other follicles were in various stages of connective tissue atresia. The surface epithelium was cylindrical, of medium size. The gland duct epithelium was similar. There were frequent mitoses present in both; the connective tissue is fibrillar. At one place the uterus had been thickened. Here the connective tissue was somewhat edematous, the connective tissue nuclei were small; there was a mitosis present in a connective tissue cell at this place. In this case abortion had probably taken place at the thickened place. In No. 105 corpora lutea had been

burnt three days, twenty hours after copulation; twenty and one half hours later incisions were made into the uterus and fourteen days, seventeen hours after copulation, ovaries and uterus were examined. There were mature follicles present in the otherwise typical ovaries. The uterus was similar as in No. 270. The uterus was also similar in No. 391, where two days, seven hours after ovulation, the right ovary had been excised and one corpus luteum of the left ovary. Five days later the uterus had been incised and seventeen days after the last copulation ovaries and uterus were examined. No mature follicles were found in the ovaries.

In one case (No. 687) the corpora lutea had been extirpated seven days, seven hours after copulation and ten days later the uterus and ovaries were examined. In the uterus two pregnancies were found; the mucosa of the uterus above and below the decidua was poor in nuclei and fibrillar. In the ovaries there were mature follicles, but no new ovulation had as yet taken place.

We may conclude that in cases in which after an early extirpation of the corpora lutea no new ovulation takes place, the condition of the uterus does not undergo any marked changes.

As we stated previously in the majority of cases in which within the first week after ovulation the corpora lutea had been cut out with scissors a new ovulation takes place between the eleventh and sixteenth day after ovulation. If we examine the uterus in these animals we find the typical structure which we might expect in accordance with the time since the last ovulation.

(1) No. 669. Forty-nine hours after last copulation, extirpation of the corpora lutea. Examination of the ovaries and uterus twenty days after the last copulation. Examination of the ovaries revealed that an ovulation had taken place about seven to eight days previously; correspondingly we find in the uterus the typical condition: rather low cylindrical surface epithelium, small glands with the same epithelium, in the connective tissue of the mucosa small vesicular nuclei, pressed together; mitoses are lacking in epithelial as well as connective tissue cells.

(2) No. 772. Two days, seventeen hours after last copulation extirpate corpora lutea; examine uterus and ovaries not quite

eighteen days after copulation. Four to five days before the examination a new ovulation had taken place. The epithelium of the surface was found cylindrical of medium height without mitoses; the glands had the same epithelium; there were mitoses present in the fundi of the glands. The connective tissue of the mucosa showed a layer of vesicular nuclei; it has a somewhat myxoid character and contained many mitoses. This condition of the uterus was in accordance with the time of the last ovulation.

(3) No. 773. Two days, eight hours after copulation, extirpate the corpora lutea; sixteen days later examine uterus and ovaries. Ovulation had taken place about seven days previously. Surface epithelium and glands of uterus show cuboidal or cylindrical epithelium of medium height; there are no mitoses present. In the connective tissue there are still a large number of proliferated cells, but only one mitosis is visible in the connective tissue under the epithelium. This corresponds to the picture of the uterus about seven to eight days after copulation.

(4) No. 779. Six days after copulation extirpate corpora lutea. Twelve days later examine uterus and ovaries. The ovaries show that rupture had taken place about four days previously. Surface epithelium and gland cells are cylindrical, relatively high, there are many mitoses in the gland fundi; they are lacking elsewhere in the epithelium. Small mononuclear cells migrate through the epithelium; the connective tissue cells are swollen and are densest under the surface epithelium; they form a distinct cell-layer; there are mitoses in these connective tissue cells.

(5) No. 785. Six days, seventeen hours after copulation, extirpate corpora lutea; twelve days later examine uterus and ovaries. The ovaries show that a new ovulation had taken place five to six days previously. There was a cuboidal-cylindrical surface epithelium of the uterus without mitoses. The epithelium of the glands was somewhat higher and also without mitoses. There is a cellular connective tissue under the epithelium with mitoses; underneath the epithelium the nuclei are more densely packed; somewhat further down the tissue is more edematous. The glands and vessels run at right angles to the connective tissue layer.

(6) No. 755. One day, nineteen hours after copulation, left uterus ligatured; extirpate one corpus luteum in left ovary; six days later extirpate one corpus luteum of the right ovary. Examination of ovaries and uterus eighteen days after copulation. Pregnancy was not present. A new ovulation had taken place approximately two days previously, to judge from the condition of the ovaries. The uterus showed high cylindrical epithelium of the surface and of the gland ducts; in both many mitoses were present; some polynuclear leucocytes are in the lumen of some glands. In the connective tissue there are small nuclei present without mitoses. Some cells migrate through the surface epithelium.

We see in these as well as in other similar cases which we need not mention here, a parellelism between the condition of the ovaries and the uterus. An experimentally accelerated ovulation leads to the onset of a new cycle in the uterine mucosa.

IV. The Uterus of Pregnant Guinea-Pigs in which After Excision of the Corpora Lutea a New Ovulation had Taken Place.

As we stated in former papers during pregnancy no new ovulation takes place in the ovary of the guinea-pig.[1] However, after excision of the corpora lutea within the first week after copulation in a considerable number of cases, a new ovulation takes place notwithstanding the presence of pregnancy.[2] We saw that if in a non-pregnant guinea-pig an accelerated ovulation takes place after an early extirpation of the corpora lutea, the uterine mucosa undergoes the typical changes characteristic of the stage of the sexual cycle at which the examination had been made. It is different, if a new ovulation takes place in pregnant guinea-pigs, as the following observations demonstrate:

(1) No. 748. Twenty-one hours after copulation ligature and cut left tube at juncture with uterus, cut out one corpus luteum in the left ovary. Seven days after copulation, cut out one corpus

[1] "The Cyclic Changes in the Uterus of the Guinea-Pig," *Journal of Morphology*, Vol. 22, p. 37, 1911; *Virchows Arch.*, Vol. 206, 1911.

[2] *Deutsche Med. Wochenschrift*, 1911, No. 1.

luteum of the right ovary. Examination eighteen days after previous copulation shows the presence of embryo and placenta in the right horn of the uterus.

In the left ovary we found young corpora lutea in which a part of the central cavity was still present, while its periphery had been filled by connective tissue; there were small and small to medium-sized good follicles and many follicles in connective tissue atresia. Both uteri show low cuboidal cylindrical epithelium of the surface with vacuoles and narrow glands. The surface epithelium and occasionally the gland ducts near the surface contain some mitoses. The rest of the glands and the connective tissue are without mitoses. The connective tissue is fibrillar and without a cell-layer. Ovulation had taken place approximately three to four days previous to examination (fourteen to fifteen days after copulation). The uterus is typical for pregnancy, but does not show the usual cyclic changes.

(2) No. 752. Ligature left tube near junction with uterus, cut out one corpus luteum from left ovary thirty-three hours after copulation. Seven days after copulation cut out two corpora lutea from the right ovary; examined eighteen days after copulation; pregnancy in right horn of uterus. In the left ovary there were three new corpora lutea, in which the central cavity had been filled by connective tissue; one of the corpora lutea contained a retained ovum in the central cavity; there were small and medium-sized good follicles and various follicles in connective tissue atresia; many follicles were in the last stage of connective tissue atresia. There were some atretic yellow bodies, the remnants of former corpora lutea. The right ovary is similar. A new ovulation had taken place about four to five days previously (thirteen to fourteen days after previous copulation). In the uterus the cyclic changes are absent: we see low cuboidal or low cylindrical surface epithelium with vacuoles which contain chromatin particles and a number of mitoses, narrow glands without mitoses and fibrillar connective tissue rich in nuclei without mitoses. No cell-layer had been formed. The structure of the uterus is the same on the pregnant and non-pregnant side.

(3) No. 780. Six days, six hours after copulation, extirpate

two corpora lutea in left, one corpus luteum in right ovary. Examination ten days later showed the presence of one pregnancy in each horn. The ovaries contained good small and almost medium-sized follicles. There was present one small to medium-sized follicle with a partial, slowly progressing degeneration of the granulosa; there are, furthermore, follicles in various stages of connective tissue atresia. In the right ovary there are two young corpora lutea in which one half to two thirds of the central cavity had been filled by connective tissue. Ovulation had taken place four to five days previously (eleven to twelve days after the previous copulation). The uterus showed low cuboidal epithelium with vacuoles and some mitoses, small glands without mitoses. The connective tissue is fibrillar and contains small nuclei; no mitoses are present in the connective tissue. Cyclic changes in the uterus are absent; the uterus is characteristic of a pregnant guinea-pig.

(4) No. 781. Seven days, five and one half hours, extirpate two corpora lutea in right, one corpus luteum in left ovary. Examination eleven days later; two pregnancies in right horn of uterus; no pregnancy in left horn. In both ovaries there were new corpora lutea in which the central cavity had been filled with connective tissue. The condition of the ovaries indicated a new ovulation about four to five days previously (thirteen to fourteen days after copulation). The uterus shows the same condition as in the former animals (Nos. 748, 752, 780). The epithelium was low, the surface epithelium contained mitoses. The connective tissue did not contain a cell-layer and was without mitoses.

(5) No. 786. Seven days, five hours after copulation, extirpate one corpus luteum in each ovary. Nine days later examination showed one pregnancy in the right horn of the uterus. In both ovaries there were young corpora lutea in which connective tissue began to grow in the periphery of the central cavity; there were small good follicles and follicles in early-medium and late connective tissue atresia. Ovulation had taken place approximately two days previously (fourteen days after copulation). The uterus showed the same structure as in the previous specimens; but the surface epithelium did not contain

mitoses. Vacuoles were however present in small cuboidal cylindricall cells.

(6) No. 789. Seven days, twelve and a half hours after copulation extirpate two corpora lutea in right, one corpus luteum in left ovary. Examination ten days later shows one pregnancy in right, none in left horn. Both ovaries showed young corpora lutea in which the central cavity had been partly or entirely filled by connective tissue. In one of the young corpora lutea an ovum had been retained. There were small and medium-sized good follicles and many follicles in connective tissue atresia. Ovulation had taken place about four to five days previously, about thirteen days after the copulation. The left uterus showed cuboidal epithelium of surface and low epithelium of glands, with only very few mitoses in the surface epithelium; there were occasionally some small vacuoles in the epithelium. The connective tissue was edematous. The blood vessels contained many leucocytes and occasionally a mitosis can be seen in an endothelial cell. Here again the typical uterine changes are absent.

(7) No. 989. Six days, eighteen hours, after copulation excise one corpus luteum in left ovary, excise the entire right ovary without corpora lutea. Examination eleven days later showed one pregnancy in the left horn of the uterus and a young corpus luteum in the left ovary. The center of the corpus luteum was filled with loose connective tissue. There were small and small-medium sized good follicles and many follicles in a medium and late stage of connective tissue atresia. Rupture had taken place about three to four days previously (about fourteen to fifteen days after copulation). The right uterus showed cuboidal-cylindrical epithelium which at some places was somewhat higher. It contained some vacuoles with chromatic material and very few mitoses; the glands were cuboidal-cylindrical and without mitoses. The connective tissue is fibrillar, without mitoses. Cyclic changes are absent.

(8) No. 988. Five days, nineteen and a half hours after copulation excise one corpus luteum in right ovary and extirpate the whole left ovary with two corpora lutea. Examination ten days later. One pregnancy in right horn of uterus. In the right ovary there were young corpora lutea in which the central cavity had

been filled with connective tissue. There were present small and large good follicles. In a few large follicles there was just a beginning of granulosa degeneration. There were many follicles in a late stage of connective tissue degeneration. A new ovulation had taken place about six to seven days previously (eleven to twelve days after the previous copulation). The uterus on both sides showed the absence of a cell layer and of mitoses in the connective tissue which was fibrillar. The surface epithelium was cuboidal or cuboidal-cylindrical, and contained a number of mitoses. The glands showed low epithelium without mitoses.

(9) No. 979. Three days, four hours after copulation excise piece of right uterus. Six days, four hours after copulation excise one corpus luteum in right ovary, extirpate the whole left ovary (with two corpora lutea). Examination twelve days later showed three pregnancies in the left horn. In the right ovary three new corpora lutea had formed not quite three days previously, about fifteen and a half days after the previous copulation. The remaining uterus on the right side showed cuboidal or low cylindrical surface epithelium with a few vacuoles and some mitoses. The glands show cylindrical shape and are at some places higher and contain mitoses. At one place there is more cellular connective tissue with some mitoses in the connective tissue cells present. At this place there had evidently a cut been made at the time of the first operation and it is probable that the abnormality which we find here has been caused either through this cut, or perhaps through an ovum that began to penetrate here into the mucosa but did not continue to develop. However that may be, the changes which we find at one place in the mucosa do not correspond to the stage of the sexual cycle in which the animal was at the time.

(10) No. 987. Five days, nineteen hours after copulation, extirpate right ovary with two corpora lutea, excise one corpus luteum in the left ovary. Examination twelve days later showed two pregnancies in the right horn, apparently none in the left. But microscopic examination disclosed that at one place in the left horn, the ovum had also inserted itself, caused the production of a small decidua, but that the ovum had found difficulty in developing,—perhaps the decidual reaction being not strong

enough. In the left ovary, there were three new corpora lutea present, an ovulation having taken place approximately four to five days previously (thirteen to fourteen days after copulation). The uterus showed low cuboidal or low cylindrical surface epithelium with a few mitoses at some places, glands with cuboidal-cylindrical, not very high cells, without mitoses, fibrillar connective tissue in the mucosa without mitoses, with exception of one place where evidently the ovum had fixed itself and called forth the development of a small decidua with mitotic divisions in the small decidual cells. The typical cyclic changes of the uterus are therefore absent.

(11) No. 967. Three days, three and a half hours after copulation, excise two corpora lutea in the left and three corpora lutea in the right ovary. Examination fifteen days later showed one embryo and placenta in the right horn of the uterus. The left horn appeared thickened. In one ovary we found one, in the other three corpora lutea; their cavity is filled with connective tissue. There are already large good follicles formed, but no granulosa degeneration has as yet taken place. New ovulation occurred about six days previously (twelve and a half days after copulation). The uterus showed at various places the typical structure of the pregnant uterus without formation of a cell-layer. Cyclic changes did not take place in the uterus during pregnancy.

(12) No. 968. Three days, thirteen hours and a half after copulation excise three corpora lutea in the left and one corpus luteum in the right ovary. Examination eighteen and a half days after copulation showed three pregnancies in the right and one small one in the upper part of the left horn. In one ovary two new corpora lutea had been formed about seven or eight days previously (ten to eleven days after the previous copulation). The mucosa of the uterus presented the typical picture of a pregnant uterus.

(13) No. 866. Seven days after copulation excise several corpora lutea both in the left and right ovary. Examination eleven days later. Abortion was in progress in both horns of the uterus. A new ovulation had taken place in one of the ovaries about two to three days previously. The cavity of the

corpora lutea was partly filled by connective tissue. In the uterine mucosa processes of regeneration took place after abortion, polynuclear leucocytes migrated through the mucosa.

(14) No. 673. Four days, two and a half hours after copulation, one corpus luteum was excised in each ovary. At the examination fourteen days later one pregnancy was found in the right horn; in the left horn there was a dilated part. In the left ovary about three to four days previously two follicles had ruptured (fourteen to fifteen days after the previous copulation; new corpora lutea had formed). In the left uterus we found cuboidal to cylindrical epithelium of the surface without mitoses; some epithelium was raised by edematous fluid; the gland ducts were drawn out; the connective tissue cells in the mucosa showed many mitoses. It is possible that an abortion had taken place in the left horn of the uterus and that this complication may explain the cell proliferation in the connective tissue.

(15) No. 1219. Three days, nineteen hours after copulation, one corpus luteum was excised in each ovary. Examination ten days later showed two pregnancies in left, one in right horn of the uterus. In the ovaries we found good follicles of small to medium size, many follicles are in a condition of early or medium connective tissue atresia. In one ovary there is a young corpus luteum with a central cavity; some blood vessels begin to grow into the corpus luteum. Ovulation had taken place about one to two days previously (about twelve to thirteen days after copulation). In the uterus we find cuboidal or cuboidal-cylindrical surface epithelium in which there are vacuoles caused by immigrated cells. There are some rare mitoses in the surface epithelium. The epithelium of the glands is low cuboidal or cuboidal to cylindrical and is without mitoses. The connective tissue is fibrillar and contains small nuclei; no mitoses are present. The early pregnancy prevents the cyclic changes in the uterus.

We see, therefore, that during pregnancy the typical cyclic changes as we described them above do not take place. Instead we find on the whole the characteristic structure of the pregnant uterus. There are two or three specimens in which some doubt about the interpretation of the structural condition of the uterus

might arise. It is probable that in those cases secondary changes, due to embedding of an ovum which was not followed by normal embryonic development, had taken place in the mucosa. Only in one case there might be some doubt as to whether the typical cyclic changes had not taken place in the uterus notwithstanding the presence of pregnancy.

As to the way in which pregnancy influences the uterine mucosa, it is probable that the activity of the living decidual cells prevents the proliferation of the uterine mucosa. It cannot be a mechanical effect of pull on the uterine wall thourgh the ovisac because the uterus of a nonpregnant horn behaves in a similar manner as the pregnant horn. If ovulation takes place after an experimentally produced placentoma has become necrotic the typical cyclic changes of the uterus follow ovulation, as in the following experiments:

No. 1152. Six days, twenty and one half hours after copulation, cuts were made into the uterine wall; examination nineteen days later (twenty-five days, twenty and a half hours after copulation). In the ovaries we found just ruptured follicles. There were present corpora lutea which were beginning to become vacuolar and to shrink (the corpora lutea of the former generation). Small good follicles as well as follicles with granulosa degeneration into which connective tissue began to grow were seen. These conditions indicated that ovulation had taken place within the previous twenty-four hours. The left uterus above the nectoric placentoma showed high cylindrical epithelium with a hyaline basal area. At some places the epithelium is lower as a result of pressure. The glands have high or medium cylindrical epithelium. There are some dilated gland ducts filled with polynuclear leucocytes. No mitoses are present in the surface epithelium and only a few in the gland ducts. At some places polynuclear leucocytes migrate into the surface epithelium and disintegrate into small chromatin particles. The structure of the uterus corresponds therefore to the condition found approximately within the first thirty-six hours after ovulation. The condition of the ovaries and of the uterus correspond to each other.

Also in another guinea-pig (No. 1140) the presence of necrotic placentomata in both horns of the uterus did not prevent the

development of the typical cyclic changes in the uterus. In this case cuts had been made into both horns of the uterus six days, six and a half hours after copulation. Nineteen days later, twenty-five days, eight and a half hours after copulation, we find on examination the presence of necrotic placentomata. Some small areas of the placentoma were still alive. Condition of the ovaries showed that ovulation had taken place about five days previously. Correspondingly we find in the uterine mucosa a cell layer with mitoses in connective tissue cells and some mitoses in gland fundi. The surface epithelium is cuboidal to cylindrical and contains some mitoses, perhaps as a result of the regenerative changes which are taking place at that time.

Pregnancy seems also to prevent the changes in the uterus which accompany the period of heat. This we found in guinea-pig No. 1300 in which three days, eighteen hours after copulation the thyroids had been extirpated. Examination seventeen days, nineteen and a half hours after copulation showed that this did not interfere with the development of the typical cyclic changes in the ovaries. They present the picture characteristic of this period. There are present several fully matured follicles ready to rupture, but the corpora lutea of pregnancy which do not show any sign of vacuolization and have vessels with well developed walls, prevent this rupture. We should expect under those conditions (period of time, presence of mature follicles) in a non-pregnant uterus high cylindrical epithelium of the surface and of the gland ducts, lower gland fundi, the nuclei of the connective tissue somewhat large and vesicular. Instead, we find the typical picture of the uterus during pregnancy; low cuboidal epithelium of the surface with a few isolated mitoses, low glands without mitoses, fibrillar connective tissue with small nuclei. At some places transudate raises the surface epithelium from the underlying connective tissue. Is it not probable that extirpation of the thyroids prevents the uterine changes accompanying heat because we observed the occurrence of these changes in another guinea-pig in which the thyroid had been removed previously. We may, therefore, conclude that the presence of pregnancy prevents those changes in the uterus which accompany the period of heat. And it appears probable that pregnancy prevents also the psychical changes characteristic of the state of heat.

V. The Influence of Extirpation of the Corpora Lutea on the Duration of the Cyclic Period.

I have previously shown that the duration of the sexual period in the mammalian female depends upon the duration of life of the corpus luteum.[1] The corpus luteum prevents a new ovulation and the onset of a new sexual cycle.

In our former experiments some operative interference had usually taken place in the case of the control guinea-pigs as well as in those in which the corpora lutea had been excised. It was therefore necessary to determine the length of the sexual cycle in the normal guinea-pig. This can be done by observing the time of the heat of a guinea-pig and by examining uterus and ovaries at known intervals. Under those conditions we found the length of the sexual period to be usually sixteen to eighteen or nineteen days; sometimes the new ovulation may take place as early as fifteen days after copulation. In two exceptional cases we observed the new ovulation as early as thirteen and a half to fourteen and a half days. If we allow copulation to take place and ligature the tubes at their juncture with the uterus, and in addition, the upper part of the uterus, within the first two and a half days after copulation, the new ovulation also may take place between the sixteenth and eighteenth day, but in other cases it occurs later. If we make incisions into the uterus throughout its whole length five to seven days after copulation, ovulation takes place in the majority of cases between the twentieth and thirtieth day after copulation. At the times ovulation occurs the corpora lutea of the previous sexual period have begun to degenerate and the experimentally produced placentomata have to a great extent become necrotic. The beginning degeneration of the corpora lutea is a prerequisite for the new ovulation. If, after copulation, pregnancy is permitted to take its normal course, no ovulation occurs until birth has taken place. If abortion has taken place, a new ovulation also can occur.

If we excise the corpora lutea within the first week after copulation, a new ovulation occurs in the majority of cases

[1] *Zentralblatt f. Physiologie*, Bd. XXIII., No. 3, *Deutsche Med. Wochenschrift*, 1911, No. 1.

between the tenth and fifteenth day after copulation. We observed it as early as eight and a half and nine days after copulation. Pregnancy does not prevent the early ovulation after a complete extirpation of the corpora lutea. It is only the persistence of the corpora lutea of pregnancy which prevents the ovulation. Pregnancy however prevents, as we saw, the cyclic changes in the uterus which accompany and follow ovulation. On a former occasion I suggested that embryonic structures determine the persistence of the corpora lutea during pregnancy.[1] We are at the present time testing this hypothesis experimentally.[2] If we extirpate the corpora lutea incompletely, no new ovulation takes place.

These experiments, as well as those mentioned above which have already been partly published, and which as far as they concern new problems shall be published more in detail in the near future, clear up to a great extent the mechanism of the sexual cycle.

VI. The Signs of Heat in the Guinea-Pig.[3]

(Report by Miss A. E. C. Lathrop.)[4]

"When a number of females are kept together in a large pen,

[1] *Zentralblatt f. Physiologie*, Bd. XXIV., No. 6.

[2] We produced recently experimentally an early stage of extrauterine pregnancy in a guinea-pig. The presence of some living embryonic structures and of fetal placenta did not prevent the degeneration of corpora lutea and the subsequent rupture of follicles. On the other hand the corpora lutea persisted as long as experimentally produced placentomata (structures of maternal origin) remained alive in cases in which we made incisions into the uterus six or seven days after copulation. In some of these cases fetal structures developed in these placentomata; in other cases no fetal structures could be found. It is therefore possible that the maternal placenta determines directly the life of the corpora lutea and that the embryonic structures act only indirectly by prolonging the life of the placentomata. We are testing experimentally this theory at the present time.

[3] The condition of heat in the majority of the guinea-pigs which we used was observed by the breeders, Mr. J. M. Simpson in Colwyn, Pa., and Miss A. E. C. Lathrop in Granby, Mass. Dr. O. Ishii also observed the condition of heat in a number of cases. I had an opportunity to control the correctness of these observations by the microscopic examination of ovaries and uterus. In almost all cases I found the observations of the breeders confirmed by the microscopical examination.

[4] I asked Miss A. E. C. Lathrop to observe the signs of heat in the guinea-pig and she sent a statement of her observations which, with her permission, I wish to incorporate in my paper after some slight changes have been made. She writes as above.

some will be seen sitting quietly, some eating a little, or walking about, making a small, sociable clucking noise.

"If one moves occasionally, rather majestically, among the rest, making a low, purring noise, similar to the noise of the male, she is probably in heat; if not, she soon will be. In some cases, a female guinea-pig may reach a state of excitement in which she assumes the manners of a male and attempts copulation with another guinea-pig which happens to be in heat. She, as well as the second guinea-pig that allows her to attempt the rôle of a male, will almost always be found ready for copulation.

"In many other cases, however, no such clear indications are present, but on close observation it will be seen that some female which is sitting quietly will be found to elevate slightly her hips whenever another guinea-pig happens to touch her. Again other females will show no sign of their condition, unless an active male is put among them. Those in heat take interest in the male, when he comes near them, while those not in heat or near the period of heat will kick him and hop away, making a little complaining noise when he pays attention to them. The male has no means of selecting the females which are in heat. When he has accidentally found one, he cannot find her again a moment later, but must try every female which he happens to come near to until he by chance meets again the animal in heat. Neither do the females that are in heat go to the male, but they wait, wherever they happen to be, until he comes to them. Some females will not give any indications whatever of their condition, but only when the male attempts copulation will they be found to be in heat.

"There are occasionally individuals which always appear to be in heat; at frequent intervals when not busy eating they purr and walk about like a male, but never seem to be actually ready for copulation. They resent the advances of the male at all times and yet are not sterile; when left for some months in a lot of males and females they may become pregnant.

"When one or very few females are kept in a small cage and have become relatively tame, those in heat when tickled along flanks and hips will react to the touch of the fingers, assuming a

position suitable for copulation.[1] This sign is, however, of no use when the animals are timid or when a number of the females are kept in the pen; under those conditions they pile up in a corner when one tries to touch them.

"The condition of the vulva may be of value in determining the state of the female. If a drop of mucus can be seen in the vulva, the animal is not in heat, even if acting somewhat excitedly, but it will be found in heat within a few hours. At the times of heat we find usually a little moisture or almost dried mucus at the vulva and a slight enlargment or swelling of the entrance to the vagina which may be so slight that it becomes noticeable only if the animal is compared with others.

"In some, perhaps in most cases, a day or two before heat the animal while not showing any of the above-mentioned signs, may be unusually lively and playful, and when taken in hand and brushed very lightly over pelvic region, we notice a twitching about the pelvis and especially the vulva."

VII. Effect of Extirpation of the Ovaries on the Cyclic Changes in the Uterus.

We found that extirpation of the corpora lutea shortens the sexual cycle. It does not to any noticeable extent influence the various stages in the development of the ovarian follicles, but it causes an early rupture of mature follicles. Extirpation of the corpora lutea changes however the cyclic changes in the uterus. It prevents the predecidual proliferation of the connective tissue cells of the mucosa and modifies also to some extent the cyclic changes in the uterine epithelium. It does not however prevent those changes in the uterus which accompany heat and precede ovulation. The existence of a corpus luteum is required only as far as a certain part of the cyclic changes in the uterus is concerned; certain other changes (those preceding ovulation) are in all probability in a similar manner as ovulation itself inhibited by the existence of a functionating corpus luteum. However, if we extirpate both ovaries instead of the corpora lutea, all cyclic changes in the uterus come to a standstill.

[1] A similar observation had been previously communicated to me by Mr. J. M. Simpson.

Several months after castration, the wall of the uterus is thin; the surface epithelium is cuboidal or low cylindrical. There are mitoses present in the surface epithelium; their number varies. The glands are small and without mitoses; the connective tissue of the mucosa is fibrillar, contains small nuclei and is somewhat richer in nuclei directly beneath the epithelium than in the deeper parts of the mucosa. Two to three weeks after castration we find also low cuboidal or low cylindrical epithelium covering the mucosa of the uterus; no mitoses are present in the gland cells which are all small. The connective tissue is fibrillar and the connective tissue nuclei are small and without mitoses.

We see that after total extirpation of the ovaries the mucosa of the uterus becomes similar to the mucosa of the pregnant uterus in a non-pregnant horn. Gradually all the layers of the uterine wall seem to become thinner.

If we extirpate the ovaries within the first twenty-four hours after copulation and examine the uterine mucosa six or seven days after copulation we find the surface epithelium usually low cuboidal or cylindrical; it may, however, be somewhat higher; there are mitoses present in the surface epithelium; the glands have low epithelium and are usually without mitoses. In the connective tissue no cell-layer developed and no mitoses are visible. We find, therefore, here a similar result as after extirpation of the corpora lutea, namely, inhibition of the connective tissue proliferation and at the same time continued mitotic proliferation in the surface epithelium, which is lacking at this period in the normal uterine cycle. The extirpation of the ovaries acts in this respect principally through the lack of the corpora lutea which castration entails. In one case, however, in which fresh corpora lutea of the guinea-pigs were injected into a guinea-pig which had been castrated sixteen and a half hours after copulation, five days after castration a few mitoses were found in the connective tissue of the mucosa. We must leave it at present undecided whether the injection of the corpus luteum substance produced this result.

If we extirpate within the first twenty-four hours following copulation the ovaries and make incisions into the uterus six to seven days after copulation we find on examination ten days

after the last operation the absence of placentomata in the uterus. Mitoses are, however, present in the surface epithelium and sometimes also in the gland cells. Twice we found a few mitoses in the connective tissue of the mucosa which may either be myxoid in character or show the usual fibrillar character. Just as early extirpation of the corpora lutea prevents the experimental placentomata, so does extirpation of the ovaries have the same effect and we may, therefore, assume that also in this case extirpation of the ovaries is effective through the accompanying removal of the corpora lutea.

Through lack of the corpora lutea the sensitizing or preparing substance is removed which in combination with the mechanical factors causes the predecidual cell proliferation and the development of the placentomata. Under these conditions the cuts do not cause proliferation in the connective tissue of the mucosa.

If we extirpate the ovaries six to seven days after copulation and at the same time make incisions into the uterus, we may find on examination ten to thirteen days later either none (two cases), or very small placentomata (in one case in which the examination had been made thirteen days after excising the uterus). In the last mentioned case, about one half of the placentoma had become necrotic, the remaining part showed a hyaline character with nuclei embedded in a homogeneous glassy matrix. At one place there were still visible in the placentoma parts of the fetal placenta in the form of syncytiomatous vessels passing into the maternal tissue and also in the surrounding fat tissue. The extirpation of the ovaries caused in this case a very incomplete and retarded development of the ovum; a similar retardation in the development of the ovum we found also in a case of extrauterine pregnancy and a similar effect may be observed after extirpation of the corpora lutea. Under these conditions the soil is evidently not favorable for the development of the ovum.

In three cases in which the ovaries were extirpated eight days or almost eight days after copulation and at the same time incisions were made into the uterus, very small placentomata developed in two cases and only a trace of a placentoma in one case. On examination eleven days after the operation, a partial

necrosis was found in the placentoma; no mitoses, or only exceptional mitoses were found. Under certain conditions we observed however in the placentoma an amitotic proliferation of nuclei which may lead to the formation of giant cells. The living parts of the placentomata were hyaline and partly vacuolar.

Extirpation of the ovaries six to eight days after copulation does not prevent, therefore, entirely the production of placentomata through incisions made into the uterus simultaneously with the castration, but it reduces very much the cell proliferation and may cause a partial early necrosis.

We found also that extirpation of the corpora lutea six to eight days after copulation led to the production of placentomata which were smaller than usual. It is however possible that extirpation of the ovaries has a still more strongly inhibiting action on the growth of the placentomata than removal of the corpora lutea.

In several other series of experiments the ovaries were extirpated at periods following the incisions into the uterus. If the incisions into the uterus were made about six days after copulation, the ovaries extirpated two days later and the examination made eight or ten days after the last operation, placentomata developed in all cases, but their size varied. In one case they were of medium size, but usually they were smaller than normal. The amount of necrosis and hemorrhage also varied; these changes could be very insignificant or they could be very considerable.

In another series incisions were made into the uterus about six days after copulation; seven days later the ovaries were extirpated and seven days after castration the examination was made. In these experiments the placentomata were therefore fourteen days old at the time of examination, and the ovaries were extirpated at a time when the placentomata had already formed. The placentomata were found almost entirely necrotic; some small living areas were present usually directly under the outer surface of the placentoma or near the mucosa,—both places where the nourishment was still better. No mitoses were seen in the living part of the placentoma.

In the surface epithelium of the mucosa and in the glands of the

uterus mitoses were found. In one case mitoses were also found in the myxoid connective tissue of the mucosa and in this case mitoses in the glands were usually present at places in the neighborhood where also the connective tissue showed mitoses. In other cases, however, mitoses were only found in the surface epithelium and glands, but not in the connective tissue. These changes in the mucosa are in all probability to be interpreted as regenerative in character, following the necrosis of the placentoma.

We may, therefore, conclude that extirpation of the ovaries does not prevent regenerative proliferation in the mucosa of the uterus; furthermore that extirpation of the ovaries shortens in all probability the life of the placentomata.

In a further series incisions were made into the uterus six to seven days after copulation; ten days later the ovaries were extirpated, and after a further lapse of five days (fifteen days after incising the uterus) the examination was made. In these cases the ovaries were therefore extirpated at a still later period and the examination was made at a somewhat earlier date after castration. The placentomata were found to a great extent necrotic; some smaller parts which were better nourished, near the mucosa and around large blood vessels were usually still alive; no mitoses were found in the living cells. Here again we find proliferative processes in the surface epithelium and in three out of five cases also in the gland ducts of the uterine mucosa near the necrotic placentomata. The gland cells in those ducts in which mitoses are found are higher. Even in the connective tissue of the mucosa there may be a few mitoses in the connective tissue cells or in the blood vessels. This mitotic proliferation must in all probability again be interpreted as regenerative in character. Castration does therefore not prevent these regenerative changes in the mucosa of the uterus.

In control experiments in which incisions were made into the uterus six to eight days after copulation but in which the ovaries were left intact, the placentomata were still alive thirteen days, and in some cases partly alive eighteen and nineteen days after the incisions had been made. In other cases they were entirely necrotic eighteen to twenty-four days after incising the uterus.

If, however, only one horn of the uterus had been incised and pregnancy proceeded in the other horn the placentomata were invariably found alive even as late as twenty-five days after incising the uterus.

We see that the function of the ovaries depends partly upon the presence of functionating corpora lutea; the latter cause the predecidual proliferation of the connective tissue of the uterine mucosa, are a necessary factor for the production of the placentomata and determine directly or indirectly at certain periods of the sexual cycle, the size, shape and mitotic proliferation of the epithelial cells of the uterine mucosa. Other parts of the changes in the uterine mucosa, namely, those accompanying or directly preceding heat are determined by other parts of the ovaries and not by the corpora lutea. They take place after extirpation of the corpora lutea; they lead indeed to the formation of new corpora lutea; they are however prevented through the extirpation of the ovaries. Which part of the ovaries is responsible for these changes characteristic of heat cannot be easily determined experimentally but can only be surmised. On a former occasion we stated the reasons why we thought it very improbable that the so-called interstitial gland of the ovary is responsible for the cyclic changes accompanying heat.[1] In the first place in the ovary of the guinea-pig there does not exist a structure deserving of the name "interstitial gland." We merely find small shrinking connective tissue cells of theca interna filling the place of lost parts of the follicles which are in process of atresia; secondly the quantity of follicles in late stages of atresia is perhaps greater five to six days after ovulation than at the period of heat, There is usually towards the end of a cyclic period a considerable number of such follicles present in the ovary, but in other cases, especially in younger animals, the number present may be relatively small.

The only structure which is, so far as we know, invariably connected with the presence of heat and which originates at the time preceding heat is the mature follicle. The granulosa cells of a mature follicle differ in some of its morphological and physiological characteristics from those of ordinary growing follicles.[2]

[1] *Centralblatt f. Physiologie*, Bd. XXV., No. 9.

[2] *Journal of Morphology*, Vol. 22, March, 1911; *Virchows Arch.*, Bd. 206, 1911.

It is therefore probable that they are in some way responsible for the changes in the uterine mucosa accompanying heat. Even if extirpation of these follicles should not prevent the occurrence of heat, this would not be conclusive evidence against this assumption inasmuch as the mature follicles had already had an opportunity at the time of extirpation to exert their influence. In a similar manner we have shown previously that extirpation of the corpora lutea five to six days after ovulation does not prevent the production of placentomata, while extirpation done one day after ovulation has a preventive effect. There still remains of course the ordinary follicles as the possible source of the energy leading to these changes in the uterine mucosa; inasmuch as they are present equally at other times of the sexual cycle, while the mature follicles are present only at certain periods and especially at the time of heat or immediately preceding heat, it is more probable that the mature follicles are responsible for these changes. Whatever the part of the ovary may be that brings about these changes, there must be added to this positive factor two negative ones, namely the absence of a fully functionating corpus luteum and of pregnancy, these conditions being necessary for the development of the normal and complete sexual cycle.

As we saw, the ovaries have still another function, namely that of maintaining the full size of the uterus. After castration various constituent parts of the uterine wall diminish in size.

VIII. Summary.

1. The cyclic changes in the epithelial as well as connective tissue structures of the uterus during heat preceding copulation and the changes following copulation are described; the influence of polynuclear leucocytes in these changes is shown. The latter are present during the first two days following copulation. The decidual cells originate from the connective tissue cells of the mucosa.

Various periods of the sexual cycle are characterized through the shape and mitotic proliferation of various epithelial and of the connective tissue cells. From the sixth to the twelfth day after ovulation mitoses are usually absent in the epithelial

structures; afterwards they begin to reappear, from the eighth day on to the end of the sexual cycle mitoses are absent in the connective tissue.

2. As long as the corpora lutea functionate, the presence of mature follicles in the ovaries does not produce those changes in the uterine mucosa which are characteristic of the period of heat.

3. During pregnancy the structure of the uterus is that of a resting organ, in which epithelial as well as connective tissue cells are small and the latter are without mitotic proliferation.

4. In animals in which heat has been observed without copulation taking place, the cyclic changes in the uterus are approximately the same as in animals in which copulation did take place, in which however pregnancy had been prevented through an early ligature of the fallopian tubes. The main difference in these two series is caused through the very marked migration of polynuclear leucocytes through the mucosa in cases in which copulation took place.

5. Ovulation is usually accompanied or directly followed by certain changes in the uterine mucosa. These changes permit us in many cases to decide whether or not in an animal in the period of heat ovulation has or has not yet taken place.

6. Extirpation of almost the whole uterus or thyroids does not prevent the occurrence of heat in the operated animals.

7. In animals which were prevented from copulating or in which the fallopian tubes were ligated soon after copulation, the period of heat occurs approximately every fifteen to nineteen days.

8. The proportion of ovarian follicles which are in the late stages of connective tissue atresia to well preserved follicles and follicles in the stage of granulosa degeneration shows two maxima, one about six to seven days after ovulation and another at the time preceding the next ovulation. Under certain conditions, however, the number of quite atretic follicles may be small at the time of heat, namely in young guinea-pigs which are in heat for the first time, and it may be relatively large at other periods of the sexual cycle in old guinea-pigs.

9. As we showed previously an early extirpation of the corpora

lutea leads in the large majority of cases to an acceleration of the next ovulation. Certain conditions, however, may prevent the early ovulation. In the latter cases the structure of the uterus corresponds to the late stage of the sexual cycle. If however a new premature ovulation is brought about through the extirpation of the corpora lutea, a new cycle starts in the uterus at the time of the accelerated ovulation. Mature follicles, or possibly other follicles, are in the absence of corpora lutea able to call forth the structural changes in the uterus accompanying heat and the rupture of the follicles and development of corpora lutea is followed by the typical uterine cyclic changes.

10. We stated previously that also during pregnancy extirpation of the corpora lutea is followed by an early ovulation. While thus pregnancy in itself does not prevent an ovulation to take place (only the corpora lutea persisting during pregnancy preventing the ovulation) pregnancy does prevent the cyclic changes of the uterus preceding, accompanying or following ovulation; during pregnancy the uterus retains its resting condition notwithstanding ovulation. This influence of pregnancy is not a mechanical one, inasmuch as it is found in the non-pregnant horn as well as in the pregnant horn in cases in which a pregnancy developed only in one horn. The presence of a necrotic placentoma or the occurrence of an abortion does not prevent the cyclic changes in the uterus that set in with ovulation.

11. If five to seven days after copulation both horns of the uterus are split lengthwise and the development of a normal pregnancy is prevented, ovulation is delayed under those conditions; it occurs between the twentieth and thirtieth day after the previous copulation, even in cases in which a developing embryo could not be seen in the placentomata growing at the site of the incisions. On the other hand in a case of an experimentally produced extrauterine pregnancy degeneration of the corpus luteum and a new ovulation were not delayed notwithstanding the presence of living embryonic (including placental structures of embryonic origin) in the animal. These observations suggest that it is primarily the life of the maternal part of the placenta which prevents the new ovulation during pregnancy and that the embryonic structures are only indirectly concerned

in this effect. We are testing this suggestion still further at the present time.

12. A description of the signs of heat is given in accordance with the observations of the breeder, Miss A. E. C. Lathrop.

13. After total extirpation of the ovaries, the uterine mucosa becomes similar to the mucosa of the pregnant uterus in a non-pregnant horn; gradually an atrophy of the different layers of the uterine wall takes place. If we extirpate the ovaries soon after ovulation the effect is the same as if the corpora lutea are extirpated at that period, as far as the predecidual proliferation in the connective tissue and the accompanying changes in the epithelium which take place between the fourth and seventh day are concerned. As far as this effect is concerned the corpus luteum is the active part of the ovaries. While, however, extirpation of the corpora lutea does not prevent those uterine changes which accompany heat and does not prevent a new sexual cycle, extirpation of the ovaries prevents all cyclic changes in the uterus.

14. Early extirpation of the corpora lutea within the first forty hours after copulation prevents in a similar manner the development of experimental placentomata as an early extirpation of the ovaries. If the ovaries are removed six to eight days after copulation and at the same time incisions are made into the uterus, either none, or only very small placentomata develop and the developing placentomata die at an earlier date than they would have done otherwise. An extirpation of the corpora lutea six to seven days after copulation also causes the developing placentomata to be smaller. If under these conditions an ovum develops in the incised uterus within the decidual tissue, the development of the ovum is usually much retarded. A similar retardation of the development of the ovum we observed in a case of experimentally produced extrauterine pregnancy in the guinea-pig. If the ovaries are extirpated two days after the making of the incisions the developing placentomata are usually smaller. Castration done at a time when the placentomata had already reached a considerable size causes an acceleration in the necrosis of the placentomata. Castration does however not prevent regenerative changes in the uterine mucosa.

15. The existence of pregnancy in one horn of the uterus prolongs markedly the life of placentomata produced in the other horn.

16. For certain phases of the cyclic changes of the uterus the presence, for other phases the absence, of a functionating corpus luteum is necessary; but certain other ovarian structures (probably mature follicles and not the so-called interstitial gland) are required for the latter phases. In addition the absence of pregnancy (and probably of living placentomata) is a condition for the typical course of the cyclic changes in the uterine mucosa.

A PRELIMINARY ACCOUNT OF THE CHROMOSOMES IN THE EMBRYOS OF ANASA TRISTIS AND DIABROTICA VITTATA.

WILLIAM E. HOY, JR.,

PRINCETON UNIVERSITY.

It was suggested to the writer, in 1912, by Prof. E. G. Conklin that a comparative study be made of the chromosomes in the somatic cells of certain insects, as contrasted with the sex cells. The first material was collected during the following summer, and included embryos of *Anasa tristis* and *Diabrotica vittata*. Material of a number of other forms, not restricted to the Insecta has since been collected and examined to some extent. The results obtained from the latter, together with a more extended account of the forms briefly described here, will be discussed at greater length in another paper.

Embryos of *Anasa tristis* were obtained covering a period of development from the incomplete blastoderm stage up to about the time of hatching. Some difficulty was experienced in obtaining the eggs of *Diabrotica vittata*, but fortunately the stages obtained extend from the second maturation division up to the segmentation of the embryo.

Anasa tristis.—In 1906, Wilson in his "Studies on Chromosomes," Part III., gave the results of his work on the spermatogenesis of *Anasa tristis*. There are twenty-one and twenty-two chromosomes in the spermatogonia and oögonia, respectively. Of the twenty-one chromosomes in the spermatogonia, twenty can be paired. There is, here, a marked differentiation in the size of the chromosomes. In each plate there are three large or macrochromosomes, and two small or microchromosomes. The unpaired or *x*-chromosome (idiochromosome; accessory chromosome) is one of these three largest chromosomes, although it cannot be exactly determined which one it is. The two microchromosomes have been termed the *m*-chromosomes. In the second maturation division the *x*-chromosome moves un-

divided to one pole so that one half the number of the spermatids receive eleven, the other half, ten chromosomes. Each spermatid also receives one *m*-chromosome.

In the oögonial group there are twenty-two chromosomes, which can be arranged in eleven pairs. Of these, four are macro- and two are microchromosomes. Wilson was able to count the chromosomes in the ovarian follicle-cells. These were found to be identical in number with the chromosomes in the oögonia. He says, however, that "not infrequently the number of chromosomes is much greater, and the same is true of the nuclei of the investing cells of the ovary, of the oviduct and the fat-body. In the male similar multiple groups are not uncommon in the interstitial and investing cells of the testis. Only in a single case have I succeeded in gaining a clear and complete view of such a group; but this one case suffices to give, with a great degree of probability, the explanation of the increased number of chromosomes. In this case every chromosome is exactly twice the oögonial number, namely 44." This figure, which he gives, is from a cell toward the periphery of a larval ovary, and shows eight macro- and four microchromosomes, twice the number of these particular chromosomes found in the oögonia. He suggests that the chromosomes have divided once without a corresponding division of the cell-body, and he thinks it probable that an increase in number of chromosomes in these particular cells is always due to a process of this kind.

Wilson also states that although he was unable to obtain perfect preparations of mitoses in other tissues, he is able to assert that in the ectodermal cells of the larva the number of chromosomes is "approximately the same as in the oögonia," and that nowhere else than in the described cases did he obtain a doubling of the number. He concludes that this multiplicity is due, perhaps, either to the fact that the cells in question are degenerating, or that they are highly specialized.

In 1910, Morrill published his observations on the chromosomes in the oögenesis, fertilization, and cleavage of certain Coreid Hemiptera, among them *Anasa*. He found that the oögonial groups in *Anasa* contain twenty-two chromosomes, including four macro- and two microchromosomes. The reduced number in

the matured egg he found to be eleven. The chromosomes in the reduced groups, either in the polar body or the egg, showed the same relative size differences as the corresponding pairs in the oögonia, and the chromosomes in the matured ova correspond, in general, to those of a sperm bearing the *x*-chromosome. The chromosome groups, which were counted in the embryonic mitoses of *Anasa* were from incomplete blastoderm stages. The embryos were found to be of two kinds: one containing twenty-one chromosomes, the other twenty-two. One case was reported of twenty-three chromosomes, but this Morrill suggests may be due to an accident in technique. The two different chromosome groups, those having twenty-one and twenty-two, correspond, respectively, to the groups found in the spermatogonia and the oögonia. There are the same size differences, and the same number of macro- and microchromosomes. He states that the chromosomes in general are more elongated in the embryonic mitoses than in those of the germ cells, which makes the task of pairing them extremely difficult, if it is at all possible, except in the case of the macrochromosomes and the two *m*-chromosomes.

The results, which the present report embodies, were obtained, in the case of *Anasa*, from a study of the somatic mitoses in several stages of development. Although mitoses are abundant in all the tissues of the embryos, few are sufficiently favorable for even tentative counts, still fewer for actual counts. In no case has it been possible to obtain more than seven counts from one individual, while three counts are more nearly the average. The chromosomes, especially in the metaphase groups, are crowded together, and the possibility of counting them at all depends chiefly on the extent of destaining.

At first some rather startling results were obtained. Some counts revealed a number as low as fourteen chromosomes in one plate, while others ran up to twenty-four in number, and there seemed to be no number characteristic of any one tissue, though the average varied around eighteen and twenty. When, however a large number of series was examined, and the exact point of optimum destaining was acquired, the results tabulated showed that the seemingly aberrant counts were wrong, and that they might be due to any of the following reasons; (1) counting early

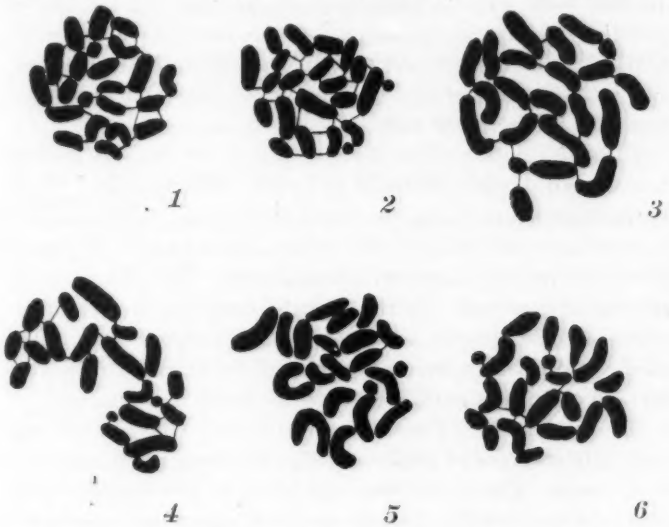
prophases, where the chromosomes lie at different levels, and are crowded and overlapped; (2) counting plates, one or several chromosomes of which lay in an adjoining section (though this does not mean that such figures are necessarily valueless); (3) some chromosomes were torn out of the section; (4) one or more chromosomes had been cut by the microtome knife, and the resulting ends or segments appeared in the next section; (5) the destaining of the slide had not progressed far enough to differentiate, one from another, some of the chromosomes lying quite close together.

Approximately one hundred groups of chromosomes from different tissues, and from a large number of embryos, have been drawn and counted. The stages, extending from the incomplete blastoderm stage up to about the time of hatching, which have been examined, are at a period of more or less high differentiation, when any chromosomal differentiation would most likely be apparent, if this is a regular concomitant of cell differentiation. The results so far show most conclusively that there is no such differentiation in the number of the chromosomes in *Anasa*, and that the embryos are of two classes as to their chromosome number, one having twenty-one, and the other twenty-two chromosomes in all the somatic cells. Moreover, the same general size differences in the chromosomes may be observed in these comparatively late stages of development as are apparent in the spermatogonia and the oögonia.

The accompanying figures serve to illustrate these points. The three upper figures (Figs. 1, 2 and 3) are from embryos having twenty-two chromosomes, and are at a stage of development where the limbs are quite elongated and the embryo as a whole has not shortened up. Figs. 1 and 2 are from the hypodermal layer of the antenna, and are from the same embryo. In both figures it will be noted that there are four chromosomes larger than the others, though this difference in size is perhaps clearer in Fig. 2. In each of the figures, again, the two *m*-chromosomes appear. Fig. 3 is from a cell in the cerebral ganglion of another embryo. These cells are much larger than any others in the embryo, consequently the chromosomes are much larger. The *m*-chromosomes are easily seen, and one pair of chromosomes is

distinctly larger than the others. The second pair of macrochromosomes is somewhat more difficult to make out.

The three lower figures (Figs. 4, 5 and 6) are from embryos with twenty-one chromosomes. Figs. 4 and 6 are from mesoderm cells of two different embryos, at a stage when the limb buds are first appearing. Fig. 5 is from a cell in the cerebral ganglion



Anasa tristis. Initial magnification of 4,800 diameters, reduced one third. Figs. 1 and 2, hypodermal layer of the antenna, 22 chromosomes. Fig. 3, cerebral ganglion cell, 22 chromosomes. Fig. 4, mesoderm cell, 21 chromosomes. Fig. 5, cerebral ganglion cell, 21 chromosomes. Fig. 6, mesoderm cell, 21 chromosomes.

of another individual, at a much later stage of development. In each of these figures the *m*-chromosomes are easily distinguishable, and it can be seen that there are two paired and one unpaired macrochromosome, particularly well shown in Fig. 4. Although no attempt has been made to pair the chromosomes, in several of the figures shown it would be quite possible to do so.

In all the cases studied the twenty-one chromosome groups generally show quite clearly three chromosomes larger than the rest, while the twenty-two chromosome groups show four such chromosomes. In most of the figures the *m*-chromosomes stand

out clearly. In a few cases it is difficult to make them out, owing to the fact that they lie very close to a larger chromosome.

Of course a difference in the general size of the chromosomes as a whole is to be found, and it is probable that no two groups would appear exactly alike. The size of the chromosomes in the metaphase groups is directly proportional to the size of the cytoplasmic body. In the metaphase groups there is a longitudinal splitting of each chromosome. In no case has a transverse division been observed here, nor has there been observed a doubling in the number of chromosomes in this phase, such as Wilson describes in the follicle cells.

Diabrotica vittata.—The spermatogenesis of *Diabrotica vittata* was worked out and published in 1907 by Miss Stevens. In the spermatogonial divisions she found twenty-one chromosomes of various sizes and shapes. The *x*-chromosome which she figures is one of the medium-sized chromosomes. The other twenty she was able to pair. In the second spermatocytic division the *x*-chromosome appears as a more or less rounded body, which passes undivided to one of the poles of the spindle. There are no such clear cut size differences as are found in *Anasa*.

The results which I have obtained from a study of the eggs and early embryos of *Diabrotica*, though somewhat meager it is true, clearly support the same conclusion as was reached in the case of *Anasa*, namely that the embryos are of two classes, one having twenty-one, and the other twenty-two chromosomes in all the mitoses.

Some clear cut figures were obtained in the maturation of the egg. The anaphase of the first maturation division revealed eleven dyads. These in turn divided, leaving eleven monads in the fully matured egg. This brings the maturation of the egg of *Diabrotica* into line with that described for other insects.

In conclusion it may be said that in neither *Anasa* nor *Diabrotica* were there any numerical differences between the gonial and the somatic chromosomes, such as have been described in the rabbit by Winiwarther, and more recently, in man by Wieman, in *Osmia cornuta* by Armbruster, and in *Diaptomus* by Krimmel.

LITERATURE CITED.

Armbruster, L.

'13 Chromosomenverhältnisse bei der Spermatogenese solitärer *Apiden*. Arch. f. Zellf., Bd. 11, H. 2.

Krimmel, O.

'10 Chromosomenverhältnisse in generativen und somatischen Mitosen bei *Diaptomus coerulens* etc. Zool. Anz., Bd. 35.

Morrill, C. V.

'10 The Chromosomes in the Oögenesis, Fertilization and Cleavage of Coreid Hemiptera. BIOL. BULL., Vol. XIX., No. 2.

Stevens, N. M.

'07 The Chromosomes in *Diabrotica vittata*, *Diabrotica soror*, and *Diabrotica 12-punctata*. Jour. Exp. Zool., Vol. 5.

Wieman, H. L.

'13 Chromosomes in Man. Am. Journ. Anat., Vol. 14, No. 4.

Wilson, E. B.

'06 Studies on Chromosomes. III. Journ. Exp. Zool., Vol. 3.

Winiwarther, H. von.

'00 Le corpuscule intermediaire et le nombre des chromosomes du Lapin. Arch. d. Biol., T. 16.

THE ECOLOGICAL IMPORTANCE OF THE RHEOTACTIC REACTION OF STREAM ISOPODS.

W. C. ALLEE,

THOMPSON BIOLOGICAL LABORATORY, WILLIAMS COLLEGE.

CONTENTS.

1. Earlier work on the rheotaxis in isopods 52
2. Survey of streams distribution of *Asellus communis* in the Chicago area .. 53
3. Relation of rheotaxis to maintainance of position in streams 55
4. Interrelations of thigmotaxis and rheotaxis 57
5. Discussion and summary 63

EARLIER WORK ON RHEOTAXIS IN ISOPODS.

Some time ago it was shown that the stream mores of the isopod *Asellus communis* (Say) is usually positive in its rheotactic reaction ('12).[1] When measured in an intermittent circular current[2] this was shown by the relatively high percentage of positive responses (79 per cent. of 1,570 trials). In a continuous straight current the same tendency was shown by the relatively long time (7 hrs., 12 min.) which stream isopods would remain positive before their ultimate reversal. Pond isopods of the same species, on the other hand, gave weak positive reaction in the intermittent current (25 per cent. of 775 trials) and there was an average of only 51 minutes of positive reaction before reversal in a continuous straight current.

The difference in the reaction of the isopods from the two habitats is closely correlated with certain natural conditions, the most important of which is the oxygen content of the water. The oxygen tension of the streams which isopods inhabit is much higher than that of the ponds. Laboratory tests show that increasing the amount of oxygen present in water makes pond isopods more positive and decreasing the oxygen tension tends to make stream isopods less positively rheotactic.

But even under uniform and favorable external conditions

[1] Numerals standing alone have reference to earlier papers by the author.

[2] For details of the method see Allee, '12, p. 276; '13, p. 261.

not all stream isopods are positive all the time and some individuals show more daily variations than others ('13*a*). These variations have been analyzed by means of the resistance of isopods to potassium cyanide ('14) and by the rate of carbon dioxide production accompanying different degrees of positive rheotactic reactions (Allee and Tashiro, '14). These analyses indicate clearly that the degree of positiveness of the rheotactic reaction of an isopod is closely correlated with its rate of metabolic activity; that a highly positive rheotactic reaction is correlated with a relatively rapid rate of metabolic activity and that when either internal or external conditions interfere with the metabolic rate of the isopod, the rheotactic reaction becomes less positive.

There are certain periods in the life cycle of stream isopods when the positiveness of the rheotactic reaction is markedly reduced. These may or may not be due to the metabolic conditions obtaining at the time. The young isopod either disregards or is very erratic in its response to a water current until it is about a month old ('12). Later at the frequently recurring molting periods the same tendency is shown for about five hours preceding and following the molt ('13, '13*a*). The breeding period is also a period of weakened positive rheotactic response ('12) in which the stream isopods lose about two-thirds of their positiveness.

The breeding season begins rather later in the stream than in the pond mores ('11) but it is in full swing in April and early May. By late May the adults become rare but the season's young may be taken one third or more grown. These young isopods are at first indefinite in their rheotactic reactions. They become highly positive more rapidly in water which has a high oxygen tension but they will come to be highly positively rheotactic even if confined to pools that have a low oxygen content ('12).

Survey of Stream Distribution of *Asellus communis* in the Chicago Area.

I have studied the local distribution of *Asellus communis* in the Chicago area only (Shelford, '11, '13, maps). Most of the stream isopods used in the experimental studies summarized

above were taken from the County Line Creek, which flows into Lake Michigan near Glencoe, Illinois. About three sixteenths of a mile above its mouth the creek branches and its total length, including tributaries, is about half a mile. This is a physiographically young stream in the intermittent rapids, permanent pool stage with numerous stones scattered over its bed. It is ranked by Shelford ('13) as the third in age of his series of twenty-six stations in the study of stream ecology.

Stream isopods also occur in the following streams:

1. Pettibone Creek, a large stream north of the County Line Creek. Shelford ranks this as next older than the County Line Creek in his physiographic series. In many ways it resembles the younger stream but is more permanent and contains less sewage. The isopods are much less abundant.

2. North Branch of the Chicago River at Des Plaines, Illinois. Shelford ranks this as a "moderately swift medium-sized stream" and as station 16 in his stream series of 26 stations. When visited in August, 1911, the stream was reduced to a series of pools without connection, hence the part visited was younger than Thorn Creek. The isopods were found among the numerous stones on the bottom of the pools.

3. Thorn Creek at Thornton, Illinois. Shelford lists this as station 12 in his stream series. This is a rapid permanent stream about 20 feet wide at ordinary flow. There is much sewage present from Chicago Heights. The isopods here are scarce, being confined to (*a*) protruding roots under overhanging banks; (*b*) under the edge of rocks in the wide shallow riffles; (*c*) in embayments or among relatively permanent catches of drift. This stream represents about the largest, oldest stream in which the stream mores of these isopods can maintain themselves. Isopods may occur in very old streams of the sluggish base-level type, as in the Fox River at Cary, Illinois, but the part of the stream in which they are found is ecologically equivalent to a mature pond (Station 35, p. 34; Shelford '13).

From this study of the streams which contain isopods it was found that the stream mores of *Asellus communis* are most numerous in young streams of the intermittent rapids, permanent pool type; especially those that contain some sewage, and that

they become more rare as the streams become larger until they are entirely lacking in many streams of the Thorn Creek stage of development. Also they are not found in streams that do not afford a good chance for lodgment.

The distribution of isopods shows a decided seasonal variation in the County Line Creek which is the only one of the streams listed above which has been sufficiently studied to give conclusive evidence on this point. In the dryer periods they are of course limited to the pools. When the stream is flowing they tend to scatter over the bottom among the debris or under overhanging banks where they may congregate in large numbers clinging to protruding roots. During the summer period the isopods are often collected in large groups along the edge of the masses of leaves that have been gathered and forced together by the spring floods. During late summer and early autumn they may be easily collected by finding and securing these groups. Later in the autumn the isopods tend to occupy the whole stream, crawling over the bottom and among the new fallen leaves. In the spring they are still more widely scattered.

Relation of Rheotaxis to Maintenance of Position in Streams.

How much of this seasonal and regional distribution is determined by the rheotactic reaction *per se?*

In 1908 the entire County Line Creek was dry except the pool nearest the lake. The rainfall of the following spring was normal. Shelford ('11) found that under these conditions the fish had all been driven to the lowest pool and in 1909 only one species, the horned dace (*Semotilus atromaculatus*) had made any progress upstream and that only about three rods. In July, 1909, the isopod *A. communis*, was found in the second permanent pool from the source and was abundant a quarter of a mile from the lake.

The horned dace is much more efficient in its rheotactic reaction than is *Asellus*, hence this distribution further towards the headwaters of a stream that was dry the preceding year must must be accounted for by some other factor than rheotaxis. Stream isopods if placed on a dirt bottom in a pan of water that

is allowed to dry, will burrow into the moist mud and thus escape death for some time and it is highly probable that they were able to survive the drouth of 1908 in this manner and to take up their usual places though in less than their usual numbers with the late autumn rains.

Another line of evidence that tends to minimize the importance of rheotactic reaction in the ecology of stream isopods is found in the analysis of the reaction itself. In 6,630 discontinuous minute reaction periods 95 per cent. of which resulted in positive rheotactic responses the average rate of movement was 76 cm. per minute. The current in which these trials were made averaged 472.5 cm. per minute, hence this represents the response to a mild current. When exposed to the continuous action of a straight current of about this strength the most positive stream isopods reversed their reaction and became negative in less than twenty-four hours. As the rate of the current was increased in laboratory tests the isopods came to rest and clung to the bottom, making no effort to advance. This reaction was given before the current became as strong as that found in parts of the County Line Creek.

These observations show that positive rheotaxis may enable isopods to maintain their position for a time in parts of a stream having a weak current but after continued exposure or in strong currents the clinging reaction becomes the more important one. This is in accord with the fact that isopods are only found in streams having abundant places for support (p. 55). If the clinging reaction is essential for the most positively rheotactic adults, it must be much more so for the early juvenile stages and for adults in the breeding season or during the molting period when the positive rheotactic reaction is either weakened or entirely absent.

There is no correlation between the seasonal variation in the positiveness of the rheotactic reaction and the seasonal distribution, as described for the County Line Creek, but the distribution can be fully accounted for by the seasonal variation in places of lodgment and surfaces that give a good foothold for crawling, that is to say in the seasonal changes in the distribution of leaves in the stream. Thus in seasonal as in regional dis-

tribution thigmotaxis seems to be of primary importance rather than rheotaxis.

Interrelations of Thigmotaxis and Rheotaxis.

In testing for interrelations between the rheotactic and thigmotactic reactions of isopods that might prove of ecological importance the following methods were employed: Isopods were tested individually in an intermittent circular current for their rheotactic reaction; immediately following this the individuals were tested in a straight trough to find how strong a water current they could resist.

These tests were made in a wooden trough painted with water proof paint over which fine quartz sand had been sprinkled while the paint was soft in order to give a good surface for the isopods. The sides met at the bottom forming a V-shaped trough with an enclosed angle of about thirty degrees. The trough was 75 cm. long and was used with a pitch of 8 cm. The current was produced by tap water.

In making the test the isopod was oriented as desired in a very small stream of water. A rubber tube 5 mm. in diameter connected with the tap faucet was introduced 5 cm. above the isopod. The faucet valve was gradually opened until the current swept the isopod off its feet. This occurred in less than a minute so that fatigue did not affect the result. Then the flow in cubic centimeters was measured. In the early work five successive tests were averaged to give the resistance of the isopod for the day but this was found to be too severe and in the later work only one test was made per day.

In the weak current the isopods would usually start to move in the direction in which they were headed ('12) although those oriented negatively attempted to turn more often than the others. They usually continued moving until the current became fairly strong, then stopped and clung to the boards in the angle at the bottom of the trough.

When headed upstream the head was placed close to the bottom as movement ceased the head and front of the thorax made an arch which kept the water running over the relatively smooth convex back of the isopod. The anterior legs are used more in

clinging than the posterior ones, which act as braces. When thus clamped the isopods withstood a flow as high as 6,600 c.c. per minute under the conditions of the experiment. Sometimes they failed to clamp when headed positively but this occurred less often than in any other position. The speed of the current successfully resisted appeared to fully equal or surpass the most rapid flow at usual stream levels in those streams in which the isopods are abundant.

When oriented negatively the isopods may clamp and withstand a strong current (up to 3,959 c.c. per minute as compared with 6,600 c.c. per minute when oriented positively). In this reaction the abdomen is bent downward much as is the head when the animal is turned in the opposite direction, but it is more easily forced up than the head and hence this is a less efficient method for clinging. Isopods fail to clamp more often under these conditions than when headed positively. This may be due to the difference in length of the posterior and anterior legs. The latter are short and can take more active part in the clamping reaction than can the longer posterior ones. The failure to clamp allows the negatively oriented isopods to be more often swept off their feet by a relatively slow current.

Comparative tests of the average clinging efficiency of isopods oriented positively and negatively support the evidence given by the maximum clinging ability under the two conditions. The results of these tests which were made under identical conditions are given in Table I. The orientation of the isopod was alternated to equalize a possible fatigue effect. In every case except isopod No. 170 the strength of the current resisted when the isopods were headed upstream was approximately double that necessary to sweep the negatively headed isopods off their feet. An average of 64 tests on 10 different isopods shows that a flow of 2,534 (± 72) c.c. per minute was required to sweep a positively oriented isopod off its feet while 1,385 (± 92.6) caused those headed negatively to lose their footing. In other words a positively oriented isopod is approximately twice as efficient in maintaining its position in a stream as is one oriented negatively.

There are indications that isopods could maintain themselves

TABLE I.

Showing the effect of orientation upon the strength of current in which isopods can maintain their position. These tests were made under entirely identical conditions throughout. In testing each animal, one to three trials were made with the isopod facing in one direction then the orientation was reversed and an equal number of trials were made. This was done to equalize possible fatigue effects. For further details of the method used see p. 57.

Oriented Positively.

Trials.	Identification No.									
	168	169	170	171[1]	172	21	22	23	168	173
1	4,750	1,300	2,750	2,750	4,350	3,000	2,100	1,140	4,033	3,450
2	3,350	2,400	2,000	2,500	2,850	2,160	1,800	1,500	2,200	2,100
3	4,000	3,400	1,800	2,000	3,850	1,500	2,100	1,620	2,550	3,450
4		1,500	3,750	2,700	3,300	2,280	2,280	2,580	2,490	3,300
5		2,400	2,350	2,500	3,300	1,560	5,100	2,820	3,530	3,900
6						540	1,800	3,000	3,240	
7							2,000	2,280		
8							2,160	1,950		
9							1,800	1,800		
10							2,160	1,560		
11							2,280	2,400		
12							900	1,920		
Average .	4,033	2,200	2,550	2,490	3,530	1,973	2,207	2,072	3,007	3,240

General average of 64 trials, 2,534 (± 72)

Oriented Negatively.

Trials.	Identification No.									
	168	169	170	171[1]	172	21	22	23	168	173
1	3,300	600	3,050	850	200	1,500	2,160	1,440	1,800	200
2	1,900	600	2,500	450	300	2,040	600	1,380	1,360	200
3	200	1,400	3,950	1,900	350	1,860	360	300	2,610	200
4		2,500	1,800	1,050	2,700	1,080	420	1,200	850	2,800
5		1,700	2,650	2,750	3,600	2,280	1,920	1,260	1,430	2,800
6						1,920	2,760	360	1,240	
7							300	1,080		
8							1,920	600		
9							600	1,800		
10							300	2,520		
11							300	1,760		
12							200	600		
Average .	1,800	1,360	2,610	850	1,430	1,780	987	1,192	1,547	1,240

General average of 64 trials, 1385 (± 82).

in quite strong currents when headed at right angles to them. In doing this an isopod used the feet of one side as anchors and swung below them. This method of maintenance gave erratic results with the apparatus used. It also brought the isopod

[1] Copulating pair.

into different relations with the current since when oriented transversely part of the body would be near the surface instead of the whole body being at the bottom, as is the case when the animals are oriented either positively or negatively. Since with the present apparatus it was impossible to obtain comparable results and since stream isopods usually go either positive or negative this method of maintenance will not be discussed further.

The data so far presented make it evident that the rheotactic reaction *per se* is not sufficient to account for the maintenance of position of stream isopods but that it has an effect upon the clinging ability of isopods in the current that is very important. In other words the movement accomplished in the rheotactic reaction of isopods is of much less importance in maintaining their position in streams than is the sign of the rheotactic reaction, which becomes of prime importance by means of its influence upon the clinging ability of isopods. Since the sign of the rheotactic reaction is closely correlated with the metabolic rate of isopods it follows that an isopod with a relatively high rate of metabolism would give positive rheotactic responses and hence be better able to maintain its position in a stream than an isopod having a relatively low rate of metabolism which would be accompanied by negative or indefinite rheotactic reactions.

It has already been noted that there are two periods in the natural life of adult stream isopods when the usual highly positive rheotactic reaction is lowered, namely: the breeding season and the shorter but more frequently recurring molting period. Both of these times are the more critical in that the clinging ability is, or may be, directly affected by them. During and immediately after molting an isopod is easily swept off its feet by a water current and in the breeding season at the time of copulation the pairs have their clinging ability reduced, especially when oriented negatively (cf. No. 171, in Table I., p. 59). After the female liberates the young and before the brood pouch is molted, a bubble of air may get into it and an isopod thus afflicted finds it almost impossible to remain on the bottom even in quiet water. These floaters often occur in the laboratory and have been observed to some extent in nature. Thus both the molting period and the breeding season reduce the maintenance efficiency

of stream isopods, directly by decreasing the clinging ability and indirectly by decreasing tendency to orient positively in the current which in turn affects the clinging ability of the isopod.

The rheotactic reaction has been shown to vary with the changing metabolic state of the isopod (Allee and Tashiro, '14). The clinging power of positively oriented isopods also varies to a limited extent, with the degree of positiveness of the rheotactic reaction. This is shown by the data exhibited in Tables II. and III. In Table II, the average rheotactic response

TABLE II.

Showing the relation between low and high clinging ability of positively oriented isopods and the rheotactic reaction. For details of methods see pp. 57 and 61.

Responses Compared.	No. Trials.	Ave. Current Resisted.	Average Rheotactic Reaction.				Ave. Efficiency.[3]
			+	−	a	o	
Current resisted 3,500 c.c. per minute or more.....	65	4,418	78 (±2.6)	18	4	0	2.7
Current resisted, 2,000 c.c. per minute or less.....	99	1,497	57 (±2.6)	23	10	10	2.3

of isopods that resisted a current of 3,500 c.c. per minute or more is compared with that given by isopods that were swept off their feet by a current of 2,000 c.c. per minute or less. The isopods with the higher clinging power averaged 78 per cent. positive in their rheotactic reactions and they did not fail to give the rheotactic response when stimulated. On the other hand the isopods with the poorer clinging power averaged 57 per cent.

[3] In order to have a method for measuring and recording the efficiency of movement of isopods in the current during rheotactic tests the following arbitrary standard was adopted, which represents numerically the distance covered in a minute's reaction period. The following values were applied to the numbers:

0, no reaction.

1, slight movement.

2, any response between 1 and 3.

3, progress one third around the pan positively (approximately 27 cm.) or two thirds, negative (54 cm.).

4, progress two thirds around the pan positive, or one and one third negative.

5, progress once around the pan positive, or twice around negative.

6, any distance over 5.

Experiments showed that isopods oriented negatively covered twice the distance per leg movement as did those oriented positively.

positive and gave no response to the current in 10 per cent. of the trials. Thus the rheotactic reaction averaged 21 per cent. less positive when the isopods had the lower clinging power. Since this is four times the probable error it must be accepted as strongly indicating that isopods with high clinging ability when positively oriented, tend to give a higher percentage of positive rheotactic reactions than those with low clinging power.

The converse data of this relationship is shown in Table III,. which lists the average current strength necessary to sweep isopods off their feet that had given: (*a*) highly positive rheotactic reactions; (*b*) low positive rheotactic reactions; (*c*) a high percentage of failures to respond to the current. In all cases the only variation known was in the metabolic state of the isopods.

TABLE III.

Showing the relation between clinging ability of positively oriented isopods accompanying a rheotactic reaction of 60 per cent. or more positive; 40 per cent. or less positive: and 40 per cent. or more no reaction. For details of method see pp. 57 and 62.

Responses Compared.	No. Trials.	Ave. Rheotactic Reaction.				Ave. Efficiency.[4]	Ave. Current Resisted.
		+	−	a	o		
Rheotaxis 60 per cent. or more positive	160	90	6	4	0	2.7	2,898 (±66)
Rheotaxis 40 per cent. or less positive	87	13	45	22	20	2.1	2,230 (±81)
Rheotaxis 40 per cent. or more no reaction	21	5	1	15	79	0.24	1,990 (±109.5)

The data presented in Table III., show that isopods having a 13 per cent. positive rheotactic reaction gave 23 per cent. less clinging ability than those that were 90 per cent. positive in their rheotactic reactions. With isopods that gave a high degree of inaction in the rheotactic tests there was a further reduction in the clinging power. The change in the strength of current successfully resisted is not at all proportional to the variation in the rheotactic reaction with which it is compared but the difference in clinging power accompanying high and low positiveness of the rheotactic reaction is 4.5 times the probable error and is therefore significant.

[4] For explanation of average efficiency see footnote 3, p. 61.

Not enough data has been collected to warrant any conclusions being drawn regarding the possible relationship between the clinging ability of isopods and their metabolic rate as measured either by their resistance to potassium cyanide or their carbon dioxide production. However the data given in Tables II. and III. show that there is a variation in the clinging power of positively oriented isopods accompanying more or less closely the variation in the positiveness of the rheotactic reaction. Since the degree of positiveness of this reaction depends on the rate of metabolism (*loc. cit.*) it seems fair to conclude that there is some correlation between the physiological states of isopods and their ability to maintain themselves in a water current. This means that a decrease in the metabolic activities of isopods not only causes a decrease in the positiveness of the rheotactic reactions and hence indirectly decreases the clinging power of isopods in a current (p. 60) but that it also directly decreases the clinging power.

Discussion.

In all probability the sum total of the reactions of an organism largely determines its distribution (Shelford and Allee, '13; Wells, '14). With different animals in different environments now one reaction or group of reactions may be the more important and now another. It is obvious that the maintenance of position is of primary importance in the distribution of stream animals and it is likewise obvious that this must be done either by some means of clinging or by the rheotactic reaction or by a combination of the two. Bryozoa show the maximum development of the clinging reaction while certain fishes as *Notropis* show a maximum development of the rheotactic response since they rarely rest on the bottom (Shelford and Allee, '13). A study of maintenance methods of stream animals would probably establish a completely graded series between these two extremes. In such a graded series all crawling animals, to which group the isopods belong, would of necessity depend a great deal upon their ability of clinging to the substratum to maintain themselves.

To what extent positive orientation in the rheotactic response increases the clinging efficiency of crawling stream animals other than isopods is a matter for experimentation. Judging solely from

structure it would seem entirely probable that such long bodied animals as *Hydropsyche* larvæ, dragon-fly or may-fly nymphs and stone-fly nymphs would show this correlation to some extent. Among fishes, the bottom resting darting fishes of the type represented by *Boleosoma* and *Etheostoma* should also show this correlation. Further experimentation on these forms would probably demonstrate, as the experiments here reported for isopods, that the sign of the rheotactic reaction continues to be of prime importance in the problem of maintenance of position in streams long after the rheotactic movements have ceased to be significant.

How much of this behavior of stream isopods is an adaptation to life in streams? In order to get the problem clearly stated perhaps a recapitulation of relationships is permissible. By means of their highly positive rheotactic reactions the stream isopods are better enabled to maintain themselves in the stream environment through the interaction of the rheotactic and thigmotactic reactions. This tendency to give positive rheotactic reactions is much weaker in pond isopods and can be modified within certain bounds in both pond and stream mores by varying the oxygen tension of the water. Therefore it seems that this is not a specific adaptation for stream habitation which the isopods exhibit, but rather that adaptation (perhaps better called property), of all living matter namely, irritability (Mathews, '13). Beyond that, the isopod is no more adapted to the stream environment by reason of its positive rheotaxis than the stream is adapted to the isopod by virtue of presenting the complex of environmental conditions (high oxygen and low carbon dioxide tension) best calculated to call forth and maintain positive rheotaxis. Environmental conditions automatically cause an isopod to give those responses which fit it to maintain its position in the stream.

Summary.

1. The distribution of the isopod *Asellus communis* cannot be accounted for by its rheotactic reaction alone, but can be accounted for by the interacting thigmotactic and rheotactic reactions.

2. The movements given in rheotactic reactions of isopods are

of secondary importance in their maintenance of position in streams.

3. The sign of the rheotactic reaction is of primary importance in this regard in that an isopod oriented positively can withstand approximately twice the current strength of one oriented in the opposite direction.

4. The clinging ability of positively oriented isopods varies somewhat with the degree of positiveness of the rheotactic reaction which in turn is closely correlated with the metabolic conditions of the isopods.

5. The molting period and breeding season are especially important in the ecology of stream isopods in that they directly decrease the clinging ability and indirectly affect it by lowering the tendency to give positive rheotactic reactions.

6. Beyond possessing irritable protoplasm isopods are no more adapted to the stream environment by reason of their positive rheotaxis than the stream is adapted to the isopod by virtue of presenting the complex of environmental conditions best calculated to call forth and maintain positive rheotaxis.

LITERATURE CITED.

Allee, W. C.

'11 Seasonal Succession in Old Forest Ponds. Trans. Ill. Acad. Sci., Vol. 4, pp. 126–131.

'12 An Experimental Analysis of the Relation Between Physiological States and Rheotaxis in Isopoda. Journ. Exp. Zoöl., Vol. 13, pp. 269–344.

'13 The Effect of Molting on Rheotaxis in Isopods. Science, N.S., Vol. 37, pp. 882–3.

'13a Further Studies on Physiological States and Rheotaxis in Isopoda. Journ. Exp. Zoöl., Vol. 15, pp. 257–295.

'14 Certain Relations between Rheotaxis in Isopods and their Survival Time in Potassium Cyanide. Journ. Exp. Zoöl., Vol. 16, pp. 397–412.

Allee, W. C. and Tashiro, Shiro.

'14 Some Relations between Rheotaxis and the Rate of Carbon Dioxide Production of Isopods. Journ. An. Beh., Vol. 4, pp. 202–214.

Mathews, A. P.

'13 Adaptation from the Point of View of the Physiologist. Am. Nat., Vol. 47, pp. 90–105.

Henderson, L. J.

'13 The Fitness of the Environment. An Inquiry into the Biological Fitness of the Environment. MacMillan, New York, 317 pp.

Shelford, V. E.

'11 Ecological Succession, I. Stream Fishes and the Method of Physiographic Analysis. BIOL. BULL., Vol. 21, pp. 9–36.

'13 Animal Communities in Temperate America. Univ. of Chicago press. 362 pp.

Shelford, V. E. and Allee, W. C.

'13 The Reactions of Fishes to Gradients of Dissolved Atmospheric Gases. Journ. Exp. Zoöl., Vol. 14, pp. 207–266.

Wells, Morris M.

'13 The Resistance of Fishes to Different Concentrations and Combinations of Oxygen and Carbon Dioxide. BIOL. BULL., Vol. 25, pp. 323–347.